Table of Contents

All Disease Areas and Platforms: Highlights ................................................................. 1

Disease Area Asthma and Allergy: Abstract No. 001 – 038 .......................................... 12
Disease Area Chronic Obstructive Pulmonary Disease (COPD): Abstract No. 101 – 122 52
Disease Area Cystic Fibrosis: Abstract No. 201 – 225 .................................................. 75
Disease Area Pneumonia and Acute Lung Injury: Abstract No. 301 – 331 ..................... 101
Disease Area Diffuse Parenchymal Lung Disease (DPLD): Abstract No. 401 - 457 .......... 133
Disease Area Pulmonary Hypertension: Abstract No. 501 – 532 .................................. 191
Disease Area End-Stage Lung Disease: Abstract No. 601 – 612 ................................. 224
Disease Area Lung Cancer: Abstract No. 701 – 738 .................................................... 237
Biobanking and Data Management Platform: Hands-On Session ............................... 276
Biobanking and Data Management Platform: Abstract No. 801 – 809 ....................... 278
Imaging Platform: Abstract No. 901 – 917 ................................................................. 288

Author Index.................................................................................................................. 306
All Disease Areas and Platforms: Highlights
Abstract No. A

Serological neo-epitope extracellular matrix related markers reflecting collagen or elastin degradation are elevated in asthma

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Asthma is a chronic inflammatory disease, characterized by symptoms including increased mucus production, reversible airway obstruction and lung inflammation. Extracellular matrix remodeling is associated with the release of ECM protein fragments (neo-epitopes) to the circulation. We sought to investigate the relationship between serological assessment of ECM remodeling markers (neo-epitopes) and the level of symptoms in a mouse model of asthma and the characterize these markers in the adult participants of the German Center of Lung Research All Age Asthma (ALLIANCE) Cohort.

Methods: Balb/C mice were sensitized to ovalbumin (OVA) (i.p.), acute exacerbations were provoked by i.n. instillation of poly-cytidylic-inosinic acid. Markers of matrix metalloproteinase (MMP) degraded collagen type I (C1M), type III (C3M), type IV (C4M), elastin (ELM7) and laminin (LAMa5) were assessed in serum. In the ALLIANCE cohort (n=233) biomarkers reflecting ECM remodeling were assessed serologically at baseline including 86 severe asthmatics, 106 mild-moderate asthmatics and 41 gender- and age-matched healthy controls. Lung function, blood cytology and clinical symptom scores were recorded. Analysis-software: JMP13 (SAS).

Serum levels of C1M and LAMa5 individually correlated with bronchoalveolar lavage cells (BAL) in mice. Furthermore, the increase of airway resistance (C4M rs=0.42, p<0.01; C1M, rs=0.55, p<0.001), the absolute airway resistance (C1M, rs=0.51, p<0.001) and the dynamic compliance (C1M, rs=-0.47, p<0.01), were correlated with the ECM remodeling markers in mice. Additionally, C1M showed a distinct correlation with the amount of mucus producing cells (Pearson coefficient 0.60, p=0.0006). In severe asthmatics patients C1M significantly increased compared to healthy controls (p=0.0495). In severe and mild-moderate asthmatics an increase in collagen type IV degradation compared to healthy controls was found (p=0.0065 and p=0.0244), respectively.

These data suggest, that serological neo-epitope markers may be valuable tools for objectively assessing the extent of airway ECM degradation. This might have clinical implications and could optimize treatment.
Evidence for metabolic syndrome and insulin resistance in smokers with COPD from serum metabolite analysis at rest and during exercise

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Introduction: There is an ongoing demand for easily accessible biomarkers that reflect the complex nature of physiological and pathophysiological changes in COPD. It was the aim of this study to test the hypothesis that using exercise challenge as a model could potentially amplify clinically relevant biomarkers in COPD. Methods: Twenty-three smokers with COPD (GOLD 2) and 23 sex- and age-matched healthy smokers underwent up to 30-minute submaximal, constant-load exercise on two occasions separated by 4 weeks (second challenge n=19/20). Serum samples were obtained before, 5 minutes after the start, at the end of exercise, and following a recovery of 20 minutes. Data analysis was performed using a mixed effects model. Metabolite level was modeled as a function of the disease and time point interaction, gender, and an individual-specific random intercept. Results: The median (IQR) exercise time of COPD smokers was 10.0 (4.0) in the first and 10.0 (8.0) minutes in the second challenge. Healthy smokers were able to exercise for 22.0 (16.0) and 26.5 (14.5) minutes. Lactate levels were comparable between groups at rest, during exercise (3-4 fold increase) and declining during recovery. Significantly higher glucose levels were observed in COPD at rest and during exercise. Fourteen of 21 amino acids (AA) showed lower levels at rest in COPD. During exercise the branched chain AA declined in healthy smokers, but remained stable in COPD smokers. Free carnitine (C0) and the acyl-carnitines C16 and C18:1 were significantly enhanced in COPD at rest. C0, C2, C3 and C4 carnitines showed lower increases during exercise in COPD smokers. Conclusion: Higher serum glucose levels, evidence for impaired AA uptake to muscle tissue during exercise and a shift of the energy metabolism to enhanced consumption of lipids are compatible with a metabolic syndrome and insulin resistance in COPD smokers.
Abstract No. C

**Randomized Double-blind Controlled Pilot Study on Safety and Efficacy of Hypertonic Saline as Preventive Inhalation Therapy in Infants with CF (PRESIS)**


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Early diagnosis by NBS has created a unique window of opportunity for early therapeutic interventions in patients with CF. Preclinical studies demonstrate that preventive hydration therapies such as inhaled hypertonic saline (HS) effectively reduce airway mucus plugging and associated mortality in mice with CF-like lung disease (Graeber, AJRCMB 2013). However, safety and efficacy of preventive hydration therapy in infants with CF remains unknown. The aim of this study was, therefore, to determine safety and initial efficacy of preventive inhalation of 6% HS versus 0.9% isotonic saline (IS) in infants with CF in a multicenter, randomized, double-blind, controlled trial performed across 5 study sites in Germany. 42 infants CF were included (mean age 96±28d) of whom 40 patients (95.2%) completed the one year-study protocol. Safety as the primary endpoint was met with no difference between number, kind, severity or distribution of AE or SAE between both treatment groups. Compliance was comparable in both groups with 70-100% of performed inhalations. Secondary efficacy endpoints revealed i) an overall low number of pulmonary exacerbations (~1/py) with no difference between groups; ii) no significant differences in lung structure and perfusion detected by chest MRI; iii) a decrease in SF6-LCI in the HS group while LCI was stable in the IS group (change in LCI P<0.05); iv) health-related QoL was on a comparable high level in both groups; v) increase in weight was significantly better in HS group (P<0.05) after 1 year; and vi) no difference in number or time to first acquisition of CF pathogens (e.g., Pseudomonas aeruginosa) between groups. Therefore, inhalation with hypertonic saline in infants starting during the first 4 months of life is as safe as inhalation with isotonic saline. Secondary endpoints point in the direction of a higher efficacy of HS underlining the usefulness of an early start of mucolytic therapy.
Abstract No. D

Influenza A virus relocalizes Na,K-ATPase in vitro and in vivo to support its replication – impact on lung injury and alveolar edema clearance

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Objective

Influenza A virus (IAV) infection may cause the Acute Respiratory Distress Syndrome (ARDS). We previously defined that the epithelial Na,K-ATPase (NKA), a major driving force of alveolar fluid clearance and thus disease outcome, was downregulated in IAV infection by a paracrine mechanism. Here, we investigated if a direct virus-host cell interaction could further affect NKA.

Results

IAV infection results in a marked decrease of NKA expression in neighbouring alveolar epithelial cells (AEC). However, in infected AEC ex vivo as well as in vivo, NKA was retargeted from the basolateral to the apical cell membrane whilst other markers of cell polarity retained their localization. Furthermore, NKA was incorporated in newly formed virions, and inhibition of NKA activity by ouabain ablated IAV replication, implying a crucial role for NKA in the viral life cycle. Indeed, NKA relocalization was specifically induced by the viral M2 protein, which could be demonstrated to interact with the NKA by co-immunoprecipitation experiments.

Conclusion

We provide evidence that NKA is relocalized to the apical cell membrane upon an interaction with the viral M2 protein. Additionally, NKA activity is a necessary prerequisite for IAV replication. We therefore suggest that targeting NKA expression and localization will both improve edema clearance and reduce viral replication and in IAV-induced ARDS.
Abstract No. E

Potential role of NLRP3 inflammasome and IL-1β pathway in granuloma generation of sarcoidosis.

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Introduction: Sarcoidosis is an inflammatory lung disease characterized by granuloma formation. The NLRP3 inflammasome plays an important role in the production of biologically active IL-1β and IL-18.

Objectives: We investigated the role of the NLRP3 inflammasome in granuloma formation in sarcoidosis and the effect of potential treatment strategies inhibiting the activation of this pathway.

Methods: We analyzed BAL cells of 36 patients with sarcoidosis and 37 healthy volunteers (HV). NLRP3 inflammasome activity of AM was measured either by caspase-1p20 expression using Western Blot or IL-1β production (ELISA). Relative NLRP3/GAPDH and miRNA-223/U6 expression was detected by RT-PCR. NLRP3 inflammasome activation was induced by priming with LPS (1µg/ml) and subsequent activation with either ATP (1nM) or Nigericin (NIG 10µM) for 6h. We used the TDM-granuloma mouse model to evaluate lung granuloma burden in miR-223 KO and NLRP3 KO mice as well as to test the effects of the NLRP3 pathway inhibitor MCC950.

Results: We found a significant increase in both spontaneous and LPS+NIG stimulated IL-1β production of AM derived from sarcoid patients compared to control AM (p=0.0003, p<0.0005 respectively). After specific stimulation with LPS + NIG we also detected an increase in caspase-1p20 expression of AM from sarcoid patients compared to HD. Furthermore, we found a significant increase in NLRP3/GAPDH mRNA (p=0.0023) and a decrease in miR-223/U6 (p=0.0002) relative expression levels in sorted AM of sarcoid patients compared to HD. Compared to WT increased granuloma formation in lungs of miR-223 KO mice were detected, while NLRP3 KO mice had significantly less granuloma. Mice treated with the NLRP3 inhibitor MCC950 had significantly decreased pulmonary granuloma burden.

Conclusion: In AM of sarcoid patients is the NLRP3 inflammasome chronically activated which may be related to low miR-223 levels. The novel NLRP3 inhibitor MCC950 may be a future treatment option in sarcoidosis.
Abstract No. F

Mitochondrial complex IV subunit 4 isoform 2 dependent release of superoxide is essential for acute pulmonary oxygen sensing

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Rationale: Hypoxic pulmonary vasoconstriction (HPV) which matches perfusion to ventilation is essential to avoid life-threatening hypoxemia. Hypoxia-induced mitochondrial superoxide release has been suggested to trigger HPV, but also chronic hypoxia-induced pulmonary hypertension (PH) leading to right heart insufficiency. We thus aimed to identify the role of mitochondrial superoxide release in HPV and hypoxia-induced PH and the underlying release mechanism.

Methods and results: HPV, but not chronic hypoxia-induced PH, could be attenuated by application of mitochondrial targeted antioxidants, e.g. MitoQ. Accordingly, superoxide release determined by electron spin resonance spectroscopy was increased in pulmonary arterial smooth muscle cells (PASMCs) incubated in acute, but not chronic hypoxia. Isolated ventilated and perfused lungs from mice lacking the subunit 4 isoform 2 of mitochondrial complex IV (Cox4i2-/-) lacked acute HPV and the hypoxia-induced increase of superoxide in PASMCs. Mitochondrial hyperpolarization, which can promote mitochondrial superoxide release, was detected during acute hypoxia in wild type (WT) but not in Cox4i2-/- PASMCs. Downstream signaling determined by patch clamp measurements showed decreased hypoxia-induced cellular membrane depolarization in Cox4i2-/- PASMCs compared to WT PASMCs, which could be normalized in mutant PASMCs by application of hydrogen peroxide. Chronic hypoxia-induced PH and pulmonary vascular remodeling, by contrast, were not or only slightly affected by Cox4i2 deficiency, respectively.

Conclusion: Acute pulmonary oxygen sensing is triggered by a Cox4i2-dependent mitochondrial hyperpolarization and release of mitochondrial superoxide which contributes to cellular membrane depolarization and HPV.
Abstract No. G

Systemic treg levels are associated with functional treg differences and correlate with the immunological risk in lung transplant recipients

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Background: Previously, we showed that preoperative Treg-levels are associated with the immunological risk after lung transplantation (LTX). However, it is unknown, whether functional differences exist in correlation to the Treg-frequency. Hence, Tregs were correlated with the occurrence of acute rejection (AR) and chronic lung allograft dysfunction (CLAD). Tregs were further differentiated into memory (mTreg), resting (rTreg) and activated (aTreg) subsets. Methods: In 100 recipients (m/f: 54/46, age: 53 ± 1y, LAS: 48 ± 2) before (day 0) and after (day 7, 14, 21, 90, 180, 270 and 365) LTX, Tregs (CD3+/CD4+/CD25+/FoxP3+) and subsets (mTreg:CD39+; rTreg:CD45RA+; aTreg:CD45RALow/-) were analyzed via Flow-Cytometry. Based on Treg-levels day 0, a low (n=25, lower quartile, ≤ 1.92% of CD4+) intermediate (n=50, 50% percentile, 1.93%-5.71% of CD4+) and high Treg-group (n=25, upper quartile, ≥5.72% of CD4+) were defined. Patients treated with steroids due to biopsy proven (A≥1/ B≥1) and/or clinical rejection (FEV1-loss) were defined positive for AR (n = 28). CLAD was defined according to ISHLT. Data are given as mean ± SEM. Results: Treg-quartiles remained stable over time and showed significant differences between the groups until day 270. There was a clear trend for a reduced AR rate (16% vs. 32% vs. 28%) in the high- compared to the intermediate- and low-Treg groups. An increased rate for suspected CLAD (low:40% vs. high:12%) was noted. In the high Treg-group increased aTregs (day 0:93±1% vs. “low”:83±2%) and reduced rTregs (day 0:7±1% vs. “low”:17±2%) were found, significantly different until day 180. In parallel, mTregs remained highest in the same time (high-Treg). Conclusion: We confirmed that pre-LTX Treg-quartile classification identifies an individual pattern with stable Treg-levels over time. Recipients with low Tregs carried the highest immunological risk, which was associated with increased rTregs and reduced aTregs, while continuously high Treg levels appeared protective, showing the opposite functional Treg pattern.
EMoLung-Concept: Monitoring of NSCLC patients in liquid biopsies and exhaled breath condensates

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For patients with NSCLC stage I-IIIa, surgery is the recommended method of choice. Currently, recommendations for follow-up visits are 3-6 months within the first 3 years after resection. To improve early detection of recurrence in curatively resected NSCLC patients, epigenetic analysis of liquid biopsies and RNA isoforms in exhaled breath condensates (EBC) will be performed in addition to the regularly conducted radiological imaging.

In a pilot study, DNA methylation data of surgical specimen, biopsies and liquid biopsies were combined to generate a panel of CpG allowing simultaneously analysis using next generation DNA sequencing approaches (NGS). In addition, molecular analysis of distinct mRNAs in EBCs revealed to be an applicable method for the detection of lung cancer. Blood samples and EBCs will be collected at 3 different time points:

- before surgical resection
- 3 months after surgical resection
- at time of recurrence

Supplementary blood and EBC sample collections will be performed 12, 18 and 24 months after surgery in order to generate corresponding control samples. In addition, one cohort including patients with no recurrence will be composed to generate a control group. The follow-up visits will be performed every 3 months within the first year and every 6 months within the second year after surgical resection. All of the five DZL sites will recruit patients with NSCLC stage I-IIIa, finally reaching 240 patients.

At least, clinical data, epigenetic data and RNA-data will be correlated to identify new potential biomarkers for the early detection of lung cancer relapse. The combination of blood based epigenetic profiling together with RNA-analysis of EBCs, might contribute to better follow-up monitoring of patients with NSCLC. The aim of this prospective clinical study is to generate a prognostic model leading to an improvement of disease control and initiation of early therapeutic intervention by using non-invasive techniques.
Abstract No. I

**Broad consent for pediatric biobanking – discussing crucial points of a template**

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The reluctance to conduct pediatric research has long mirrored the general paradigm that children need special protection as a vulnerable group. This has led to a relative lack of research in children. International recommendations now emphasise that “children and adolescents must be included in health-related research unless a good scientific reason justifies their exclusion” (CIOMS/WHO 2002, Revision 2016). Children participating in research must be protected as well as possible.

Including samples of children in biobanks for scientific purposes demands reconsidering central aspects of biobank-governance (Hens et al. 2011). Particularly thorny questions revolve around informed consent/assent of children, for example understanding and decision making in the developing child, or the proper age for assent (e.g. Hunfeld/Passchier 2012; Hens et al. 2013).

One aim of the "All-Age-Asthma-Cohort" (ALLIANCE) is the observation of children with wheezing and asthma from childhood to adulthood. To enable ethical and effective research, we considered the option of broad consent for pediatric biobanking, with the aim towards developing an ethical framework for pediatric broad consent. To the best of our knowledge, this is the first such attempt for German biobanks.

Whilst working towards the framework, we identified the following pertinent ethical issues we offer for discussion:

- Whether children as well as their parents have a right to withdraw;

- That parents have no surrogate 'right not to know' and that pediatric biobanks thus should have a clear policy about returning clinically actionable information on early onset diseases. Minors should be notified regardless of their parents' wishes, provided the findings are subject to assessments of clinical validity and utility.

- That each pediatric biobank should develop a policy for how data and samples will be handled once a pediatric participant reaches the age of majority. Two ways seem acceptable: anonymisation of samples and data, or re-contacting.
Abstract No. J

**Multicentre Standardisation of Chest MRI as Radiation-Free Outcome Measure of Lung Disease in Young Children with Cystic Fibrosis**

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**Background:** A recent single-centre study demonstrated that MRI is sensitive to detect early abnormalities in the lung and response to therapy in infants and preschool children with cystic fibrosis (CF) supporting MRI as an outcome measure of early CF lung disease. However, the feasibility of multicentre standardisation remains unknown.

**Objective:** To determine the feasibility of multicentre standardisation of chest MRI in infants and preschool children with CF.

**Methods:** A standardised chest 1.5T MRI protocol was implemented across four specialised CF centres. Following training and initiation visits, 43 infants and preschool children (mean age 3.1±1.5y, range 0-6y) with CF underwent MRI. Image quality and lung abnormalities were assessed using a standardised questionnaire and an established CF MRI score.

**Results:** MRI was successfully performed with diagnostic quality in all patients (100%). Incomplete lung coverage was observed in 6% and artefacts also in 6% of sequence acquisitions, but these were compensated by remaining sequences in all patients. The range of the MRI score in CF patients was similar across centres with a mean global MRI score of 13.4±5.7.

**Conclusion:** Our results demonstrate that multicentre standardisation of chest MRI is feasible and support its use as radiation-free outcome measure of lung disease in infants and preschool children with CF.
Disease Area Asthma and Allergy: Abstract No. 001 – 038
Regulation of chronic experimental asthma by mast cells and mast cell chymase Mcpt-4

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Allergic asthma is one of the most common chronic respiratory disease characterized by airway hyperreactivity (AHR), goblet cell hyperplasia associated with mucus hypersecretion, and inflammation of lung tissue. Activated mast cells (MCs) contribute to asthma by the release proteases such as chymases or tryptases, which may exert pro- or anti-inflammatory functions depending on the corresponding inflammatory context. Recent studies suggest a protective role for the mouse chymase Mcpt-4 (human MC chymase homologue) in a model of acute asthma. However, its role in a chronic course of the disease has not been investigated yet. In the current study we determined the function of mMCP-4 and analyzed its effects in the induction of the asthma pathology in an alum-free chronic mouse model of the disease. For this purpose, chronic inflammation was induced in mMCP-4−/− and their corresponding wild type (wt) control after sensitization with OVA by repeated challenge with the allergen for 9 weeks. In order to identify MC-specific effects in this model and to discriminate them from mMCP-4-regulated functions, disease was induced in parallel in MC-deficient mice and MC-deficient animals reconstituted with MC either derived from wt or mMCP-4−/− mice. In contrast to previous finding in acute asthma models, mMCP-4−/− mice and MC-deficient mice reconstituted with MC from wt animals. These findings argue for a functional switch of MC and mMCP-4 from acute to chronic disease. While in the acute situation MC play a disease promoting role and mMCP-4 protects mice from developing disease, in the latter condition chymase acts proinflammatory whereas MC may have a protective function.
Abstract No. 002

Cholinesterases in the respiratory tract of mice

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Acetylcholine (ACh), released by parasympathetic neurons, is the major bronchoconstrictor in the airways. Additional non-neuronal sources of ACh have been linked to epithelial remodeling, inflammation and proliferation of fibroblasts, indicating a potential functional role in the pathogenesis of allergic asthma. The extracellular life time and radius of action of ACh are controlled by cholinesterases, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). We here determined their expression and localization in the murine respiratory system and supplying innervation by RT-PCR, histo- and immunohistochemistry.

AChE is expressed in cholinergic nerve fibers in lung and trachea, defined by presence of the ACh synthesizing enzyme, ChAT (choline acetyltransferase), but not in noradrenergic fibers. These fibers coexpress chrna3, the cholinergic receptor nicotinic alpha 3 subunit. In the vagal sensory jugular-nodose ganglia providing sensory innervation to the lung and airways, we obtained evidence for non-quantal ACh release. RT-PCR revealed the expression of the AChE mRNA S-variant, and a subgroup of sensory neurons was AChE-immunoreactive. The peptidergic (calcitonin gene-related peptide) subpopulation, however, was AChE-negative, both in ganglia and in the airways and lung. BChE is mainly expressed on membranes of airway, but not vascular smooth muscle cells. In the epithelium, we obtained positive results with RT-PCR but did neither observe immunoreactive protein nor enzyme activity. Sensory and sympathetic ganglia expressed BChE-mRNA.

Our data show strong expression of cholinesterases at the airway neuromuscular functional unit, pointing towards a fine tuned regulation of cholinergic bronchoconstriction. Cholinergic effects upon sensory signaling also appear to be limited by cholinesterases. In contrast, sites of pleiotropic, trophic functions of non-neuronal ACh are less equipped with cholinesterases, in line with concept of long acting ACh at low concentrations.
Abstract No. 003

Cytokine Patterns of Upper Airways in Adult Asthma

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RATIONALE: In patients with asthma biological therapies become increasingly available. Consequently, inflammatory phenotyping (TH2 vs. Non-TH2) gets more important. Measurement of excreted cytokines in nasal lining fluid could be a novel, non-invasive method for molecular phenotyping of patients with asthma.

METHODS: In nasal lining fluid of n=85 patients with distinct clinical asthma phenotypes we measured 19 cytokines (IL-1α, IL-4, IL-5, IL-8, IL-10, IL-13, IL-17, IL-22, IL-24, IL-33, IL-37, IFN-γ, IFN-λ, Eotaxin-3, G-CSF, Periostin, SCGB1A1, TNF-α, and TSLP) by high-sensitive multi-array technology (Meso Scale Discoveries (MSD), Rockville, USA), a plate-based electro-chemiluminescence detection method. We also assessed differential cell counts in peripheral blood and induced sputum, and measured lung function.

RESULTS: In patients with asthma (mean age, 51.1±14.1 years; 52% male; mean BMI 27.2±5.4 kg/m2; mean FEV1 83.5±20.7%pred.; median ACT-score 19 [15-24], median daily ICS-dose 500µg [250-1000] Fluticasone), nasal cytokine levels were generally detectable in more than 75% of the subjects, except for IL-22, IL-24, IL-33, and TSLP with a frequency of non-detects of 69%, 59%, 60%, and 88%, respectively. Sputum eosinophils correlated with IL-4, IL-5, Eotaxin-3 and Periostin levels (p<0.05). Blood eosinophils correlated with IL-37 (p<0.05), and with less evidence with IL-5 (p=0.0505). Sputum neutrophils correlated with IL-17 and IFN-γ (p<0.05). FEV1 was associated with various TH2 vs. Non-TH2-associated cytokines (p<0.05) with correlation coefficients (Spearman’s r) between -0.278 and -0.440.

CONCLUSION: Non-invasive measurement of cytokines in nasal lining fluid of patients with asthma appears to be generally feasible for a broad spectrum of analytes. Correlations of both TH2 and non-TH2 associated cytokines with corresponding inflammatory markers of the lower airways indicate biological validity, while correlations with lung function indicates the clinical relevance of these upper airway biomarkers. Therefore, nasal lining fluid might be a valuable proxy of disease processes in the lower airways and complement inflammatory phenotyping of patients with asthma.
Abstract No. 004

**Primary bronchial epithelial cells change the extracellular vesicle (EV) – associated microRNAs secretion upon a developing goblet cell metaplasia**

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**Background**: miRNAs are critical regulators of signalling pathways and have been shown to be involved in the pathogenesis of asthma. Further, miRNAs are actively transported and secreted in extracellular vesicles (EV) for inter-cell communication (Valadi et al., Nat Cell Biol, 2007).

**Objective**: We therefore asked if the miRNA content of EVs secreted by primary human bronchial epithelial cells (NHBE) is altered upon the early development of goblet cell metaplasia.

**Methods**: NHBE cells (Lonza, Switzerland) were cultured at the air-liquid interface and treated with interleukin (IL)13 to induce goblet cell differentiation. EV-associated miRNAs were isolated from basal medium or apical surface washes and profiled by a qPCR-based approach (System Biosciences). After isolation by qEV (Izon Sciences) vesicle secretions were characterized by flow cytometry, transmission electron microscopy and western blot.

**Results**: EVs of different sizes were found in both apical surface washes as well as in the basal culture media. Apical EVs showed more CD9 but less CD63 expression than basal EVs, and the amount of CD63*CD9+ EVs increased by IL13. In total, 47 miRNAs were expressed in isolated EVs with differential expressions in apical and basal EVs at baseline. In apical EV secretions, IL13 induced miR-422a (logFC 1.76), while five miRNAs were downregulated, most prominently miR-210 (logFC -2.3). In basal EV secretions, IL13 reduced the expression of 13 miRNAs and in particular of miR-92b levels (logFC -6.46), while three miRNAs were slightly increased (i.e. miR-219-3p with a logFC 0.60).

**Conclusion**: Our results suggest that apical and basal EV populations differ from each other and might thus be targeting different cells. This distinct secretion pattern changed upon a developing goblet cell metaplasia. Thus, we speculate that a differential secretion of miRNAs contributes to early events in asthma pathogenesis and could be further used to decipher the mechanisms of airway remodelling in asthma.
Abstract No. 005

**Early production of IL-6, KC, and TNF precedes acute exacerbation of experimental asthma in mice**

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During the course of chronic disease acute asthma exacerbations develop as distinct clinical manifestations that are triggered by respiratory infection. Defined as acute episodes of progressive worsening of disease symptoms acute asthma exacerbations have a major impact on public healthcare, since they account for unscheduled visits of physicians, emergency departments or hospitalization and requirement of increased and/or systemic corticosteroids. Therefore, early detection of developing asthma exacerbations is needed that make early countermeasures possible.

We used an established mouse model of experimental asthma exacerbation to analyse the early virus-induced specific cytokine immune response in asthmatic mice. For this purpose, experimental allergic asthma was induced in female C57BL/6 mice by systemic sensitization to and challenge with ovalbumin (OVA). Acute exacerbation of the established disease was induced by intra-nasal application of the TLR-3/RIG-I ligand poly(I:C). Leukocyte numbers in the BAL, expression and production of various cytokines, chemokines and immuno-modulatory factors were analysed in different compartments 2, 4, 8, and 12 hours after poly(I:C) application.

We detected early influx of eosinophils and neutrophils increasing over time and reaching highest numbers after 12 hours. This was preceded by increased expression and release of chemokines like eotaxin and especially KC. While increase of T helper 2 (TH2) cell cytokine was relatively moderate, we found an early and steadily increasing production of type I and type III interferons and proinflammatory cytokines like interleukin (IL) 1 and tumor necrosis factor. Most interestingly, IL-6 levels in broncho-alveolar lavage, nasal lavage, and serum were already high 2 hours after poly(I:C) application and remained at even more increased levels until 12 hours.

Based on these data we suggest IL-6, TNF and KC as early markers of a developing exacerbation of experimental allergic asthma.
Abstract No. 006

**Rupintrivir reduced host immune response to RV1B infection ex vivo in HDM-sensitized lung tissue**

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Millions of asthmatic patients suffer from virus induced exacerbation mainly caused by human rhinovirus (RV). Insufficient virus elimination and inadequate immune response are assumed to be responsible for exacerbation. Therefore, in this study the impact of an asthmatic background on the anti-viral and pro-inflammatory immune response was investigated in precision-cut lung slices (PCLS) of HDM sensitized mice. Balb/c mice were sensitized and challenged with HDM or saline i.n. for 28 days. After PCLS preparation and infection with UV-inactivated RV1B (105 TCID50/mL), RV1B or RV1B in combination with rupintrivir for 48 h and cytokine secretion was assessed by MSD. HDM sensitization resulted in a TH-2 / TH-17 dominated allergic immune response present in lung tissue ex vivo. Significantly higher levels of IL-4, IL-5, IL-10, IL-17A and IP-10 were detectable in ex vivo culture of HDM lung slices. RV1B significantly upregulated anti-viral cytokines such as IFN-β (954±311 vs. 566±111 ng/mg protein) and IP-10 (61±17 vs. 40±6 pg/mg protein) in healthy and asthmatic tissue, with overall reduced levels of these cytokines in asthmatic group demonstrating an impaired immune response to RV1B. IL-25 and IL-33 remained unchanged in both groups upon viral infection compared to the corresponding UV-inactivated control. Further, RV1B infection exacerbated IL-4 (823±201 vs. 1374±340 pg/mg protein) secretion in asthmatic tissue ex vivo in comparison to the corresponding UV-control but remained unchanged in healthy tissue. Rupintrivir treatment significantly reduced cytokine secretion of IFN-α, IP-10, IL-4, IL-6 and MCP-1 in asthmatic tissue. Contrary to this, only MCP-1 levels were reduced in healthy tissue by rupintrivir treatment. The in vivo established asthmatic disease background by HDM challenge maintains in cell culture and leads to a significantly dysregulated immune response to virus infection ex vivo. Further studies are required to reveal pathways involved in the mechanisms of virus-induced exacerbation of asthma.
Abstract No. 007

**Delineating asthma phenotypes – the DZL All Age Asthma Cohort (ALLIANCE) – a progress report**

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**Background:** The DZL ALLIANCE asthma cohort aims to tackle the delineation of asthma pheno- and endotypes and their predictive value for disease course by subjecting a comprehensive asthma cohort of children and adults to extensive clinical and biomaterial interrogations. This approach allows following trajectories of disease phenotypes across age-groups with the aim to identify relevant pheno- and endotypes as well as associated biomarkers of progression and remission.

**Methods:** Since 2013 recruitment of children with asthma (> 6 years), recurrent wheeze (>6 months,<6 years) and healthy controls (>6 months, age and gender-matched) is ongoing at three study sites (ARCN, BREATH, CPC-M, expansion to five sites 2017: UGMLC, Children’s University Hospital Cologne) and extended to adults in 2014 (ARCN only). Patients attend clinical visits annually with questionnaires, in-depth lung function measurements and sampling of biomaterials. Healthy controls undergo the same work-up once.

**Results:** In total 624 children and 255 adults have been recruited (children: 228 > 6 yrs, 221 < 6 yrs, 175 healthy controls; adults: 208 patients, 47 healthy controls). Follow-up (FU) visits after 1, 2 and 3 years (FU1, FU2, FU3) have resulted in 350 FU1 (46 drop outs), 242 FU2 (13 drop outs), 140 FU3 (0 drop outs) and 10 FU4 (0 drop outs) in the pediatric and 153 FU1 (16 drop outs) and 78 FU2 (3 drop outs) in the adult arm. A web-based clinical database yields quality-controlled data sets. Analyses relating potential biomarkers (profiling of specific IgE, nasal and serum cytokines, cellular phenotyping via chip and flow cytometry) to clinical phenotypes have been performed, some of which are presented on separate posters.

**Conclusions:** Recruitment and prospective FU of nearly 900 subjects within the ALLIANCE cohort are excellent with the infrastructure for collaborative, interdisciplinary data analysis in place to allow mining of the obtained measures and assessments.
Abstract No. 008

Air-liquid interface cultures for the investigation of the role of human airway epithelium in early processes of asthma exacerbations

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Asthma is one of the most frequent chronic respiratory diseases worldwide. Acute exacerbations are mainly caused by viral infections and require the use of systemic corticosteroids or even hospitalization of the patient. These exacerbations seriously diminish quality of life of the patient but unfortunately cannot be predicted timely.

Therefore, it was the aim of this study to establish and define a human cell culture model mimicking asthma exacerbations for the investigation of early processes.

Human bronchial epithelial cells (HBECs) from Lonza were cultured under air-liquid interface (ALI) conditions for 28 days in order to build up a pseudostratified epithelium. The cells were stimulated with the T helper cell type 2 cytokine interleukin-13 (IL-13) from day 21 until day 28. Then, HBECs were treated with poly(I:C), a surrogate for replicating RNA-viruses, alone or in ALI cultures pretreated with IL-13 for three hours.

PAS-staining of paraffin sections revealed increased number of goblet cells after IL-13 stimulation. Co-stimulation with poly(I:C) did not further enhance GC number. The expression of the mucin Muc5AC and eosinophil chemotactic factor eotaxin 3 was increased in IL-13-stimulated cells. The addition of poly(I:C) did not further enhance expression levels of both, Muc5AC and eotaxin 3.

The expression of pro-inflammatory mediators, such as IL-6, IL-8 and tumor necrosis factor (TNFα), was increased in poly(I:C)-stimulated ALI-cultures. However, co-stimulation with IL-13 and poly(I:C) further enhanced the expression of IL-6, IL-8 and TNFα.

In conclusion, we propose that the human ALI cell culture model based on a combination of IL-13 treatment together with poly(I:C) as a trigger is suitable for the investigation of the impact of the airway epithelium during early processes of virus-induced asthma exacerbations.

Supported by the BMBF (DZL, Leibniz Research Consortium EXASENS)
Abstract No. 009

Measurement of volatile organic compounds (VOCs) in exhaled air of children of the DZL ALL Age Asthma Cohort (ALLIANCE)

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Rationale: Recent data suggest that asthma is a syndrome. Currently, no predictors of distinct phenotypes and future disease course exist. We aim to decode underlying mechanisms for distinct phenotypes and to discover biomarkers for phenotypes and future course by measurements of breath volatile organic compounds (VOCs) by gas chromatography-mass spectrometry (GC-MS).

Methods: As part of the multi-center DZL All Age Asthma Cohort (ALLIANCE) of wheezing or asthmatic children and asthmatic adults, deep phenotyping is performed in ‘omics’ analyses plus comprehensive clinical assessment. This setup includes measuring the ‘breathome’ by VOC analyses in children. As a cross-disease area cooperation (AA and COPD), this measurement has currently been established in one recruiting center (CPC-M). Preschool wheeze and asthma were defined per guidelines. Children inhaled pre-cleaned room air and exhaled into an aluminum reservoir tube. Breath from this reservoir was loaded onto two adsorption tubes and shipped to Hannover for GC-MS analyses. 157 VOCs were assessed in breath and room air samples.

Results: We analysed breath VOCs of 82 children (13 healthy controls, 47 children with asthma, 22 wheezers). In preliminary analyses we focused on differences between asthmatics and controls. We saw typical VOC compositions for breath (major compounds isoprene and acetone) and room air (disinfection compounds propanol and ethanol). In univariate analyses we found 29 VOCs that differ between asthmatics and controls. Within the group of asthmatics nine VOCs were related to gender and 20 VOCs were correlated to age with an r >0.40.

Conclusions: Breath VOC sampling is feasible even in young children. The ‘breathome’ may add to the ALLIANCE deep phenotyping approach. The number of breath VOCs that differ between asthmatic children and controls suggests that this non-invasive technique might be an excellent complementary measure to allow in-depth characterization of asthma phenotypes and identification of relevant biomarkers and predictors.
Abstract No. 010

**Asthma and allergic rhinitis are not associated with sleep quality in adolescence. Results from the GINIplus and LISA studies.**

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**Background:** Sleep affects performance, health and quality of life. Studies suggest that sleep quality is impaired in patients with allergic diseases. However, in most epidemiological studies, questionnaires are used for the assessment of sleep quality.

**Objective:** To investigate the association between allergic rhinitis and asthma with objectively and subjectively assessed sleep parameters in a population-based sample of adolescents.

**Methods:** In the German birth cohorts GINIplus and LISA, sleep quality was measured by one-week accelerometry. Between 2011 and 2014, 1376 subjects (mean age 15.6 years) were wearing wrist accelerometers (Actigraph GT3X) during night and filled in sleep and activity diaries every day. Sleep efficiency evaluated according to the Sadeh algorithm was used as an objective measure of sleep quality and the diary self-reported sleep quality (on a 1 to 6 scale) was taken as its subjective counterpart. Information on general sleep problems, current asthma and allergic rhinitis was assessed using questionnaires. Linear mixed-effects and cumulative link mixed-effects models were used to analyse the association between allergic diseases with daily objective and subjective sleep quality, respectively, adjusted for potential confounding variables including, sex, body mass index and season. Logistic regression models were used for sleep problems.

**Results:** Among study participants, 6.8% had asthma and 17.0% had allergic rhinitis. The mean sleep efficiency was 79.4% (SD=8.4) and the mean total sleep time 7.2 hours (SD=1.2). 13.9% of the study participants had general sleep problems, 12.6% had problems to fall asleep and 2.8% to stay asleep. The associations of current asthma and allergic rhinitis with objectively and subjectively assessed sleep quality were not statistically significant. Furthermore, no association with sleep problems has been observed.

**Conclusion:** The results of this study indicate that asthma and allergic rhinitis are not related to impaired sleep quality in adolescents, probably due to early stages of allergic diseases.
Abstract No. 011

House Dust Mite -- derived HODEs: players in the dynamics of allergic asthma?

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9-HODE and 13-HODE (9- and 13-hydroxy-octadecadienoic acid) are lipid metabolites of enzymatic oxidation of linoleic acid (LA, 18:2n-6)¹. HODEs belong to the so-called endogenous lipid mediators and are produced by different cell types in the lungs, such as bronchoalveolar cells, leukocytes, airway epithelial and vascular endothelial cells². 13-S-HODE has been shown to induce pulmonary hyperresponsiveness² and to contribute to severe asthma by causing mitochondrial dysfunction and bronchial epithelial injury³. Increased levels of 13-HODE in extracellular fluids of human atopic asthmatics were also reported³.

House dust mites (HDM) are important inductors of allergic asthma⁴. Chemically, aside from glycoproteins, HDM consists of a large variety of lipids. Lipids can initiate and/or modulate allergic reactions, either on their own or acting as adjuvants for allergens⁵. In addition, several HDM allergens bear hydrophobic domains, thus enabling them to interact with lipids⁵.

To examine the role of HDM-derived lipids in allergic asthma, lipids were extracted from Dermatophagoides pteronyssinus bodies, fractionated and characterized by gas chromatography/mass spectrometry (MS) and electrospray MS. In HDM-lipid fractions, 9-/13-HODE were detected. The fractions containing predominantly 9-/13-HODEs increased IgE-mediated degranulation of murine mast cells, induced proinflammatory cytokines and morphological changes in the human bronchial epithelial cell line Calu-3.

This study shows for the first time HDM as an exogenous source of bioactive lipids (HODEs), with a direct correlation to allergic asthma. Investigations are presently carried out on human primary effector cells to get an insight of the HODEs-allergen interactions and consequences for the development of allergic asthma.
Abstract No. 012

Analysis of the B cell compartment in adult asthmatics in the ALLIANCE cohort of the DZL

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Objective: Allergic asthma is a widespread chronic inflammatory disease of the airways but the influence of different B cell subsets, immunoglobulin isotypes and cytokines, which are crucial parts of the adaptive immune system, is not well-known. Since defects in development and function of B cells lead to allergy, we investigate the peripheral B cell compartment of adult asthmatics.

Methods: Isolated PBMCs from severe (35 males/40 females, 57±13 years), mild-to-moderate asthmatics (33 males/48 females, 49±14 years) and healthy volunteers (18 males/12 females, 51±18 years) were used to characterize B cells by flow cytometry. Plasma B cell activating factor of the TNF family (BAFF) was determined by ELISA and immunoglobulins by Luminex assay. Asthma severity was assessed according to ERS/ATS guideline, symptom control according to GINA guideline and eosinophilia was defined ≥300 eosinophils/µl blood.

Results: Severe asthmatics showed reduced CD19+ B cells compared to mild-to-moderate asthmatics and healthy volunteers (7.1,1.0-38.3 vs 13.4,2.7-37.8 vs 12.8,3.1-33.0 median, min-to-max). Transitional and naïve B cell subsets were decreased, whereas memory B cells were increased in severe asthmatics. Preliminary analyses of immunoglobulins showed increased levels of several isotypes in asthmatics compared to healthy controls. B cell developmental and differentiation factor BAFF was decreased as well as the expression of BAFF receptor in transitional and naïve B cells of severe asthmatics. Furthermore, uncontrolled, severe asthmatics showed reduced immature and naïve B cells along with decreased BAFF levels and elevated memory B cells compared to healthy subjects. Moreover, transitional and naïve B cells were diminished together with BAFF levels, but memory B cells were increased in non-eosinophilic, severe asthma.

Conclusions: Our preliminary data indicate that the B cell compartment is differently modulated in uncontrolled, severe and non-eosinophilic, severe asthma. Further analyses and correlations with clinical data are ongoing to improve characterization of the B cell compartment in asthma phenotypes.
Abstract No. 013

Smoking in adolescence and its potential effect on asthma risk in offspring - a murine model

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Introduction: A recent multicenter epidemiological study suggested that not only maternal smoking in pregnancy, but also paternal smoking in adolescence can increase the asthma risk in offspring. This was also observed when fathers had quit smoking years before the child was born. A possible explanation could be that primordial germ cells develop into spermatogonia and remain quiescent until the onset of puberty, when the spermatogonia resume further development. This developmental window could therefore be particularly susceptible to environmental insults (Svanes, Int J Epidemiol, 2017).

Objective: To develop a murine model of smoking during adolescence and investigate the outcome on offspring.

Methods: Male and female C57BL/6 mice (21-days old) were exposed to mainstream smoke for 2 weeks to 1 puff/min (6 cigarettes) and 4 weeks to 4 puffs/min (24 cigarettes) once a day (60 min) for 5 days/week, using research cigarettes 3R4F and the in-expose exposure system (SCIREQ, Canada). Thereafter, smoke-exposed animals were mated with air-controls. Offspring was analyzed at postnatal day (PND) 3 and PND21 by flow cytometry for immune development and lung function (PND21) assessed by FlexiVent system (SCIREQ, Canada).

Results: 67% of smoke-exposed males were successful in inducing pregnancy, whereas 25% of smoke-exposed females got pregnant. In the air-control group 50% of females were impregnated. Sperm count of smoke-exposed males was comparable to air-controls. In offspring body weights, organ weights and cell counts of lung and spleen were comparable to pups from air-control parents in both sexes. Preliminary analyses show that offspring from smoke-exposed mothers had a deceased inspiratory capacity compared to offspring from smoking fathers.

Conclusion: To further investigate the underlying mechanisms, we will analyze the gene expression and epigenetic changes of murine sperm cells of smoked fathers vs. control fathers. Further we will investigate whether offspring from smoke-exposed parents is more susceptible to allergic airway inflammation.
Breath VOC patterns in adult asthma patients of the ALLIANCE cohort

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Introduction: A breath test that provides information about the inflammatory phenotype of asthma patients could be a valuable tool in daily clinical practice. Electronic nose data suggest that breath volatile organic compounds (VOC) could serve as breath markers. To evaluate if breath VOC differences between clinical asthma phenotypes can be detected despite considerable noise due to environmental and lifestyle related VOCs, we analyzed breath VOC patterns in the adult arm of the ALLIANCE cohort.

Methods: In this preliminary analysis we included 111 subjects (29 active smokers). Among the non-smokers 36 had severe asthma, based on 2014 ERS/ATS guideline definition, and 46 had mild-to-moderate asthma. 37 non-smokers had high (> 3%) and 45 normal sputum eosinophil counts. Patients inhaled pre-cleaned room air and exhaled into an aluminum reservoir tube. The breath from this reservoir was loaded onto two adsorption tubes and shipped to Hannover for the analysis by GC-MS. 134 VOCs were assessed.

Results: A sufficient correlation between the simultaneously loaded adsorption tubes and the differences between actively smoking and non-smoking patients (44/134 VOCs with p<0.05) indicate that the breath collection and analysis were adequately performed. In line with others (Resp.Physiol.Neurobiol. 2006), we also found higher levels of isoprene in male subjects. No correlations between sputum eosinophils, exhaled NO or sputum neutrophils and VOC levels were found with r-values exceeding 0.30. In non-smokers we found more VOCs with differences between severe and mild asthmatics than between those with high and normal sputum eosinophils. One unidentified VOC that showed higher levels in severe asthma patients is also suspected to be COPD related (JBR 2016).

Conclusion: Some breath VOCs appear to be related to the clinical asthma classification, while within the selected breath VOCs only few and weak associations to sputum markers of airway inflammation were observed.
Abstract No. 015

**Influence of antibiotic treatment on the microbiota and immune system in murine infants – A possible intervention by a bacterial metabolite**

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Epidemiological studies have demonstrated a clear association between antibiotic treatments (AT) in early childhood and a higher risk of developing asthma later in life. For ethical reasons, it is impossible to clarify in human studies if antibiotics can contribute to asthma development by changing the patient’s microbiome but this is of high clinical relevance, especially in newborns and infants.

**Aim:** To assess the influence of AT on the mouse infant’s gut and lung microbiota, on the immune system and whether possible changes can be intervened by a bacterial metabolite (BM).

**Methods:** 14d old mice were treated on days 1-3 via oral gavage with PBS, 30 mg/kg vancomycin or 15 mg/kg clarithromycin. The BM (0.5 mmol/kg) was also administered via oral gavage during the AT and additionally on days 4-6. Lung and gut samples were collected on day 8 for microbiota analysis. Cytokine levels were measured by cytometric bead assay in bronchoalveolar lavage and culture supernatants of lung cells stimulated with CD3/CD28, LPS or PolyI:C. We further assessed T cell populations in thymus and lung by flow cytometry.

**Results:** Microbiota analysis of the gut revealed significantly reduced α-diversity, and the bacterial community composition of both antibiotic groups was significantly different (p=0.001) to the PBS- group. CD3/28-stimulated lung cells from antibiotic-treated mice secreted elevated amounts of Th2-related cytokines (IL-4, IL-5, IL-13, [IL-6]) in contrast to PBS-treated mice. This response could be reduced by treating animals with the BM additionally to the antibiotics.

**Conclusion:** The AT led to an altered gut microbiota, and we have first hints that it increased Th2 responses in the lung which might be ameliorated by BM. To confirm this, the T cell status and effects on the lung/gut microbiota are currently being analyzed. Next, we plan to evaluate the susceptibility to allergic airway inflammation in mice following AT.
Abstract No. 016

Chipcytometry based comprehensive immunophenotyping of peripheral blood mononuclear cells of the DZL ALL Age asthma cohort (ALLIANCE)

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INTRODUCTION: Development of childhood asthma is accompanied by immunological alterations. These alterations could explain disease mechanisms and serve as a chance to identify immunological diagnostic and therapeutic markers. We use chipcytometry to elucidate specific ‘immunotypes’ in pediatric and adult asthma within the DZL ALL Age Asthma cohort (ALLIANCE). AIM: The aim of our project is to use chipcytometry for a high content immunophenotyping of PBMCs in ALLIANCE Cohort. METHODOLOGY: We have applied chipcytometry for analysis of more than 350 clinical samples of ALLIANCE cohort. For an extensive surface characterization and identification of various leucocytes subpopulations (T cells, B cells, NK cells, Monocytes and Dendritic cells) within peripheral blood of the pediatric and adult study participants of the ALLIANCE cohort, we used a set of 16 biomarkers (CD123, CD14, CD11c, CD141, CD303, HLA-DR, CD3, CD19, CD172α, CD44, FceR1, CD56, CD16, CD45, MDC-1 and CD1c) on healthy, wheezing and asthmatics probands (children and adults). We subjected chipcytometry data to multi-dimensional analysis using novel bioinformatics approaches such as support vector machines and correlation models to differentiate various clinical phenotypic groups either using total PBMCs or leucocytes subpopulations. RESULTS: We here present successful and reproducible identification of various PBMCs subsets in multi-center recruited probands using chipcytometry. Preliminary analysis shows a differential expression pattern of functionally relevant biomarkers such as HLA-DR, CD172α, CD44 and FceR1 between healthy and either wheezing or established pediatric asthmatics as well as adult asthmatics. We show that a combination of novel bioinformatics approaches applied on chipcytometry data could be used to characterize ‘immunological signatures’ associated with wheezing, asthmatics or healthy children. CONCLUSION: Distinct “immunological signature” underlies various subgroups of DZL ALLIANCE cohort. We are currently in the process of creating immunological patterns associated with different clinical phenotypes of either atopic or non-atopic probands in comparison with healthy controls.
Abstract No. 017

Use of quantitative computed tomography in the assessment of post-interventional long-term changes after bronchial thermoplasty in patients with severe asthma

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Objectives

Bronchial thermoplasty (BT) can be considered in the treatment of severe asthma. We hypothesized that quantitative computed tomography (QCT) is an appropriate tool to measure long-term effects of BT on airway dimensions and air-trapping.

Methods

Paired in- and expiratory CT scans of 16 patients were acquired before and after BT. The fully automatic YACTA software calculated wall thickness (WT), wall percentage (WP), lumen area (LA) and total diameter (TD) as well as total lung volume (TLV) and air-trapping (E/I, RVC\textsubscript{856-950}, A1-A3) in a generation- and lobe-based approach, respectively.

Results

After BT WT (p=0.035) and WP (p<0.001) decreased significantly with a subsequent significant increase in LA (p=0.046). TD increased not significantly (p=0.706). After BT media TLV decreased by 68 cm\textsuperscript{3} (p=0.459), air-trapping had a significant reduction for the parameter IE (p=<0.001), RVC\textsubscript{856-950} (p=<0.001), and A1-A3 (p=<0.003, p=<0.001 and p=<0.001), respectively.

Conclusion

QCT showed post-interventional changes in patients treated with BT. Significantly reduced WT and WP with an increase in the lumen can be interpreted as direct therapeutic effects caused by a reduction in airway-smooth muscle mass and changes in neuronal innervation. QCT also showed a significant reduction in air-trapping. QCT-parameters may be used in therapy guidance and monitoring.
Abstract No. 018

One-Bead-One-Compound libraries – A new tool to detect and analyze unknown IgE and IgA targets in inflammatory human airway diseases

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IgE plays a detrimental role in development, manifestation and persistence of allergic asthma as well as systemic allergies, both diseases affecting hundreds of millions of people worldwide. In contrast, IgA guarding the mucosal surfaces supposedly could have a protective role in allergic airway diseases by capturing and neutralizing potential allergens.

Knowledge of the exact targets of these immunoglobulins in diseased patients is often limited. But this information is indispensable to combat IgE-based diseases via immunotherapy – the only curative approach available at present – and/or by boosting a protective IgA-immunity against foreign, potentially allergy-inducing materials.

To screen millions of different peptides for being potential targets of IgEs and/or IgAs from biological samples of healthy and diseased donors, we make use of so called one-bead-one-compound (OBOC) libraries. After performing a meticulous optimization strategy for the OBOC technique, we are now able to reproducibly detect defined IgE target species on single beads within a bead-library bearing >10,000 different peptides and are currently upscaling the amount of irrelevant beads species. Additionally, the IgE detection limits in this system are < 20 IU/ml hence in the physiological range of patient samples. Moreover, preliminary analyses also show promising results for IgA target detection with the OBOC technique and IgA concentrations < 100 ng/ml. Additional optimization in terms of detection reagents and experimental settings is intended to further improve this system as previously shown for IgE.

Our next steps are to set up a semi-automatized procedure for isolation and characterization of positive beads. Once established in full functionality, the screening procedure will then be used to analyze patient material with completely unknown IgE/IgA species. This shall allow us to identify new IgE and IgA targets with the goal of using them to diagnose and potentially cure patients with allergic asthma and other IgE-related chronic inflammatory disorders.
Abstract No. 019

**Predicting childhood asthma risk in farm and non-farm children**

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**Background** Farm and non-farm children differ in their risk of childhood-onset asthma. Gene-environment interactions do not seem to play a major role in these differences.

**Objectives** To compare prediction of childhood-onset asthma between farm and non-farm children based on family history of asthma and atopy, environmental, and genetic determinants.

**Methods** We used statistical learning approaches based on penalized regression and decision trees for prediction of asthma in 1707 school children from the GABRIELA study. Information on asthma diagnosis, family history, and environmental determinants was based on questionnaire data. Genetic information was either derived from a genome-wide dataset of single-nucleotide polymorphisms (SNPs) or on the GWAS catalogue (candidate SNPs). Quality of prediction was compared by receiver operating characteristics (ROC) curves.

**Results** The quality of overall prediction of childhood asthma in farm and non-farm children was moderate with an area under the ROC curve of 0.69 [0.38-0.96] and 0.63 [0.53-0.72], respectively. The variables most importantly contributing to the prediction models were sex and family history of asthma, atopy, hay fever, and eczema. For non-farm children about half of the 40 environmental variables also contributed significantly to the prediction. In contrast, environmental variables did not improve prediction in farm children. Rather prediction was improved in farm children by candidate SNPs related to *IL33* and *RAD50*.

**Conclusion** Environmental determinants may improve prediction of childhood asthma in non-farm children, whereas farm children may profit from their environmental exposures to a much larger extent. Thus farm children may predominantly experience asthma forms that are more strongly related to genetic determinants. Though gene-environment interactions may not play a major role for associations between farm exposure and asthma, prediction of asthma differs substantially between farm and non-farm children.
Abstract No. 020

Diagnostic testing beyond allergen extracts - Classification of patients by molecular phenotyping

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Background: The DZL-flag ship project “basic science” focuses on the effect of molecular structure on allergenicity with particular emphasis on sensitization and molecular phenotyping. Preliminary results on inhalant as well as food allergens have associated lipophilic allergens with more severe allergic reactions and asthma. Therefore, we aimed to investigate the impact of lipid-associated allergens, on sensitization and asthma phenotype to identify suitable biomarkers for disease development.

Methods: House dust mite (HDM) allergens Der p 1, 2, 4, 5, 7, 10, 15, 18, 20, 21 and 23 were produced recombinantly in E. coli and purified by chromatography steps in downstream processing. Thereafter, they were used to create a multiplex system for the identification of individual HDM sensitization patterns of KIRA- and adult patients. In the same way, sensitization to peanut allergens Ara h 2 (severity marker allergen) and Ara h 8 (pollen-associated food allergy) as well as to the recently identified peanut oleosins (potential severity marker, lipophilic allergens) was assessed.

Results: The established multiplex system allows for the simultaneous identification of specific IgE directed against various antigens and consequently the molecular phenotyping of patients with a broad spectrum of allergic symptoms including asthma to HDM and peanut. The detection rate of HDM sensitized KIRA-children was increased by almost 20% when compared with the routine diagnostic extracts. Asthmatic children were often sensitized to the major HDM allergens Der p 1 and Der p 2, and to a lesser degree to the lipophilic allergens Der p 5, 7, and 21. Peanut allergic patients with severe allergic symptoms and asthma were usually co-sensitized to Ara h 2 and the lipophilic oleosins. Ara h 8 sensitization was negligible.

Conclusion: Molecular phenotyping of patients beyond standard clinical tests allows a better characterization of patients and disease dynamics and, thereby, the decision towards the most effective treatment.
Abstract No. 021

**Investigation of cell-specific epigenetic and molecular regulation in different childhood asthma phenotypes in the ALLIANCE cohort.**

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**Background:** Asthma is the most common chronic airway inflammatory disease in children worldwide, with increasing prevalence. Clinically, childhood asthma is divided into two main phenotypes: allergic asthma (AA) and non-allergic asthma (NA). We have previously shown that T-cell function is different in school-age allergic versus non-allergic asthmatics and that CD4\(^+\) and CD8\(^+\) T-cells and subpopulations are critical for asthma protection (Raedler et al./Lluis et al., JACI, 2015; Twardziok et al., Clin.Imm, 2017). Furthermore, leukocyte polarization and immune activation are regulated by epigenetic and molecular processes, which are currently not well defined.

**Aim:** To investigate cell-specific epigenetic modifications (DNA methylation, histone acetylation and miRNA) and distinct molecular regulation (RNA-Seq) in peripheral blood of AA, NA and HC (healthy control) children.

**Method:** FACS-based cell sorting of PBMCs from school-age children (6-18 years) of the ALLIANCE cohort is performed in three centres. T-cells (CD4\(^+\), CD8\(^+\)), B-cells (CD19\(^+\)CD27\(^-\)/CD19\(^+\)CD27\(^+\)), monocytes (CD14\(^+\)) and mDCs (CD11c\(^+\)) will be isolated from children: HC (n=15), AA (n=15), and NA (n=10).

**Results:** To date, cells were successfully sorted of n=18 children. Purity of isolated CD4\(^+\) and CD8\(^+\) range between 98-99.9\%, CD19\(^+\)CD27\(^+\)/CD19\(^+\)CD27\(^-\) between 94-98\%, and CD11c\(^+\) between 93-96\%. Cell numbers are in average 1.5x10\(^6\) cells for CD4\(^+\), 0.8x10\(^6\) cells for CD8\(^+\), 0.1x10\(^6\) cells for CD19\(^+\)CD27\(^+\), 0.5x10\(^6\) cells for CD19\(^+\)CD27\(^-\) and 0.2x10\(^6\) cells for CD11c\(^+\). These are split for RNA-Seq, methylation, and acetylation analysis, depending on cell numbers.

**Future plan:** Following completion of recruitment, analysis will be performed in the complete batch of samples. This project shall contribute to understand whether CD4\(^+\) and CD8\(^+\) T-cell balance is epigenetically differentially regulated with distinct subsequent gene regulation in children with different asthma phenotypes, whether epigenetic and gene regulation of different T-cells, B-cells, monocytes and DCs determines the course and prediction of disease, and which epigenetic and gene regulation determines T-cell regulation in asthma phenotypes.
Abstract No. 022

Cytokine patterns in children with wheezing and asthma show specific patterns of variability over time

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Background: Asthma is now used as an umbrella term of a complex syndrome with heterogeneous clinical manifestations comprising variable clinical, cellular, and molecular phenotypes. I.e., distinct patterns of airway inflammation, mediated by T helper2 (Th2) cells, innate lymphoid cells (ILC2) and others, have been described in cohort studies. However, as asthmatic patients are recruited at different time points over a distinct period, the variability over time might be a critical confounder.

Methods: Children with either recurrent wheezing bronchitis (> 3 episodes; “Wheezers” = W) or doctor-diagnosed asthma (“Asthmatics” = A) and healthy controls (C) were recruited at three study sites (Hannover, Luebeck, Munich) as part of the ALLIANCE cohort. We used the Bio-Plex Pro™ Human Cytokine 27-Plex Assay (Biorad, #M500KCAF0Y).

Results: We enrolled n= 117 wheezers, n= 150 asthmatics and n= 67 healthy controls (n= 20 < 6 years and n= 47 > 6 years). Significant differences (p< 0.05) between asthmatics/wheezeers and controls were observed for IFN-γ (mean, ± SD; A: log IFN-γ 4.92 pg/ml ± 0.812; C 4.58 ± 0.58), IL-17A (Box-Cox-Transformation: W: 6.53 pg/ml ± 2.80; C: 8.71 ± 2.20) and IL-4 (Box-Cox-Transformation: W: 1.98 pg/ml ± 1.48; C: 4.22 ± 0.94). Seasonal effects were observed for IP-10, IL-4, IL-5, IL-13, IL-17A and Eotaxin. No differences were observed for following cytokines included in the analysis: FGF basic, G-CSF, GM-CSF, IL-1β, IL-1ra, IL-2, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p70), IL-15, MCP-1 (MCAF), MIP-1α, MIP-1β, PDGF-BB, RANTES, TNF-α and VEGF.

Conclusion: We developed statistical methods to control for seasonal effects and revealed that these methods have an impact for the interpretation of specific serum cytokines in children with asthma and/or recurrent wheeze.
Abstract No. 023

Distinguishing “asthma” phenotypes and sensitization patterns in four different mouse strains – role of the corresponding microbiome

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Background: Asthma is a heterogeneous disease encompassing several phenotypes. Currently, it is assumed that the risk to develop asthma is shaped by a complex interaction of host genetics, the host’s microbiome and early environmental influences. While genetics are fixed, the microbiome offers opportunities for therapeutic manipulation. This requires a clear understanding of the causal relationship between the microbiome and asthma susceptibility.

Aim: To establish murine models for different asthma phenotypes for later analysis of their gut and lung microbiota independent of the genetic background.

Methods: Four different mouse strains (females) were obtained from Jackson Laboratory. The stability of their microbiomes in our animal facility was assessed by 16S rRNA sequencing. Experimental asthma was induced by intranasal administration of 20 µg house dust mite (HDM; D. pteronyssinus extract, Greer, USA) three times weekly/ three weeks. Asthma phenotypes were evaluated by lung function, histology, immune cell infiltration, cytokine levels in broncho-alveolar lavage (BAL) and total and allergen specific IgE in sera.

Results: Increased airway resistance, BAL cell and eosinophil counts and changes in lung histology were observed in all HDM-treated groups versus controls. However, the mouse strains showed distinct asthma phenotypes, ranging from a purely eosinophilic, Th2 dominated phenotype to a more neutrophilic, Th17 high phenotype. While total IgE levels in serum were elevated in all strains, a preliminary analysis showed different IgE reactivities to single HDM allergens resulting in different sensitization patterns.

Conclusion: The distinct manifestations of the asthma phenotype in different mouse strains reflect known human phenotypes and offer the possibility to study the mechanistic link between different experimental asthma subphenotypes and the host microbiota on the one hand and sensitization on the other. To circumvent the influence of different genetic background of four strains, the microbiota of phenotypic extremes will next be transplanted to one strain of gnotobiotic mice.
Abstract No. 024

Interleukin 6 has a key role during virus induced exacerbation of asthma

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Viral infections of the lung are the major cause of acute asthma exacerbations. Double-stranded RNA motifs, produced during replication of respiratory viruses, can trigger immune responses via activation of Toll-like receptor 3 or RIG-I. We have previously shown that local application of the synthetic TLR-3/RIG-I activator poly(I:C) alone is sufficient to trigger exacerbation of experimental allergic asthma in mice.

This study aimed at identifying early regulatory mechanisms leading to asthma exacerbation. In a mouse model of poly(I:C) triggered exacerbation of allergic asthma is characterized by acute worsening of airway inflammation, mucus production and airway hyperresponsiveness. This was associated with increased production of proinflammatory cytokines e.g. IL-4, IL-5, IL-6, and IL-13. Interestingly, among these cytokines IL-6 levels revealed by far the earliest and the highest increases not only in broncho-alveolar lavage (BAL) fluid but also in nasal lavage (NAL) and serum. We made similar observations in air-liquid interface (ALI) cultures of primary human bronchial epithelial (hBEC) cells, one of the main target cells of respiratory viruses. Primary hBECs incubated with poly(I:C) + IL-13 displayed increased expression of IL-6 and mucus secretion compared to controls incubated with either IL-13 or poly(I:C) alone. We therefore, investigated the role of IL-6 in the pathogenesis of an acute exacerbation of experimental asthma. While, application of recombinant IL-6 instead of poly(I:C) did not to trigger an exacerbation, it was not possible to induce a poly(I:C) triggered exacerbation in animals deficient for IL-6. Thus, even if IL-6 alone is not sufficient to trigger an exacerbation these results indicate a critical role for IL-6 in poly(I:C) induced acute exacerbation of experimental asthma. This study suggests IL-6 potential target for therapy of virus induced acute asthma exacerbations.

Supported by the BMBF (DZL, Leibniz Research Consortium EXASENS)
Abstract No. 025

Influence of differently glycosylated IgE and IgG subclass Abs on allergic reactions

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It is still not predictable when IgE antibodies induce allergic reactions or when IgG antibodies induced by allergen-specific immunotherapy (AIT) protect from IgE-mediated allergic reactions or when IgG antibodies in the presence of high allergen doses induce allergic reactions by themselves. Because the effector function of IgE and IgG subclass antibodies is dependent on the type(s) of Fc N-glycosylation, we have started to analyze the antibody glycosylation pattern of different groups of untreated or AIT-treated allergic patients and the effector function of differently glycosylated antibodies in passive antibody-transferred mouse anaphylaxis models. We have already found that different adjuvants used in different AIT-protocols induce differently glycosylated IgG subclass antibodies and that differently glycosylated IgG subclass antibodies show opposite anaphylactic potential in mice. Our observations e.g. suggest that adjuvants used to promote IgG production during AIT should be selected for their ability to induce a certain IgG subclass with a certain type of IgG Fc glycosylation to abolish IgE- and IgG-mediated allergic reactions.
Abstract No. 026

The Remodeling Effect of TGF-beta and its Influence on the Respiratory E2 Epithelium

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Background: Asthma and other allergic diseases are recognized to be Th2-mediated inflammation conditions. Type-2 cytokines, most importantly IL-4, induce remodeling processes or cytokines such as periostin and collagen production. We hypothesized that an E1/E2 epithelium analogous to the Th1/Th2 cell is underlying this process. Another key remodeling factor is TGF-β, which may influence the E1/E2 differentiation or activation process.

Objectives: To investigate influence of TGF-β on the E2 differentiation.

Methods: Primary normal bronchial epithelial cells (NHBE) of six genetically independent donors were cultured in the presence of the Th2 cytokine IL-4, the growth factor TGF-β and the combination of both. RNA was harvested after 6h and subjected to Agilent single color microarray gene expression profiling (8x60K). Data was analyzed using Agilent Genespring Software.

Results: The analysis of the microarray data on stimulated NHBEs revealed 747 IL-4 highly induced genes, which includes among others GATA3, ANO1, MEIS1, and CCL26. These genes were up regulated even in the presence of TGF-β. Also periostin, which might be also involved in the airway remodeling processes, was highly expressed after IL-4 stimulation, while not affected by TGF-β. In contrast, PPARGC1B related to the glucocorticoid receptor was strongly induced by IL4 and counter regulated by TGF-β. The latter induced COL1A1 production, which however was not influenced by the presence of IL-4.

Conclusion: An E2 fingerprint induced by the type-2 key cytokine IL-4 was detectable. In contrast to IL-4, TGF-β has no major effects on airway epithelial cells and regulates only a low number of genes. Most of these genes are involved in remodeling processes, such as COL1A1. Furthermore, IL-4 induced PPARGC1B, which is supposed to influence the glucocorticoid receptor and leads to a down regulation of periostin.
Abstract No. 027

**IL-37 diminishes experimental allergic asthma by inhibiting the proinflammatory effects of IL-1**

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We have previously shown that asthmatic children display diminished production of the antiinflammatory cytokine interleukin (IL) 37. Based on our finding that local treatment with IL-37 reduces allergic airway inflammation and all hallmarks of experimental asthma in mice, we hypothesized that an impaired IL-37 production could lead to a decreased capacity to counterbalance inflammation and thus predispose towards development of chronic inflammatory diseases. Therefore, supplementation of IL-37 deficiency could represent a novel approach towards asthma therapy. Since the mode of action of IL-37 remains largely elusive, we aim at elucidating how this cytokine unfolds it regulatory effects on asthma.

Using different knock-out mouse strains we identified IL-18Rα and SIGIRR to form the so far unknown IL-37-receptor, which is expressed on all cells playing a role in asthma pathogenesis including T helper 2 (TH2) cells, antigen-presenting cells (APCs), and airway epithelial cells (AECs). Since IL-37 treatment significantly reduced the production of TH2-type cytokines and expression of the transcription factor GATA-3 in mice with experimental asthma, we investigated the effects of IL-37 on TH2 cells in-vitro. IL-37 treatment had no effect on TH2 cells restimulated via the T cell receptor. However, if TH2 cells were restimulated allergen-specifically by APCs, IL-37 significantly reduced the production of IL-4, IL-5, and IL-13 as well as of proinflammatory cytokines like IL-1. Since gene array analysis of lung tissues revealed that IL-37 treatment of animals with experimental asthma lead to differential expression of >90 genes induced by IL-1 signaling, we investigated if IL-37 could interfere with IL-1-signaling. Indeed, IL-37 treatment of AECs markedly reduced IL-1-induced expression of proinflammatory cytokines and chemokines. Further, IL-37 treatment had no therapeutic effects on experimental asthma in mice lacking IL-1R1.

In summary, we suggest that IL-37 down-regulates allergic airway inflammation and asthma hallmarks by inhibiting the proinflammatory activity of IL-1.
Abstract No. 028

**A mobile device for clinical investigation of human nasal mucosa with endoscopic mOCT**

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High resolution 3-dimensional imaging of nasal mucosa may give valuable diagnostic information on cilia function, mucus transport and drug response. Morphology and function can be measured on a tissue and cellular level.

After extensive animal and ex-vivo studies, we developed a rigid endoscope for clinical applications, which uses microscopic optical coherence tomography (mOCT) to visualize tissue volumes with a nearly isotropic resolution of less than 2 µm. Lateral field of view is 200 µm. Imaging speed can reach up to 150 B-scans per second. The endoscope uses MEMS scanner to reduce size and weight of the endoscope for a convenient application. A miniature RGB camera and an irrigation channel assure optimal imaging under clinical conditions. All technical components (supercontinuum light source, spectrometer, electronics, computer) were built into a mobile cart, which easily allows to bring the mOCT to different sites within the DZL.

The device was certified to fulfill all relevant safety standards (e.g. EN 60601, EN14971, EN 62304, EN 60825) for use in clinical studies. Electrical and laser safety are assured by using medical grade components and monitoring of the emitted laser power by specially designed electronics. Electromagnetic interference and electrical safety were certified by an accredited laboratory.

With the instrument endoscopic mOCT can be used within the DZL for imaging morphological and functional changes of the upper airways in patients. Its value for the diagnosis and follow up in different lung diseases such as asthma, allergy and cystic fibrosis will be investigated in upcoming clinical studies.
Abstract No. 029

Cytokine levels in ex vivo stimulated blood as biomarkers of asthma endotypes in the adult arm of the DZL All Age Asthma Cohort (ALLIANCE)

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Background: Asthma pathogenesis involves several types of immune and resident cells, as well as a large range of inflammatory mediators, amongst others, cytokines. We aimed at determining cytokine patterns in ex vivo stimulated blood samples among adult asthmatics of the ERA cohort and associate with disease endotypes.

Methods: Blood was collected in TruCulture blood culture systems, which contained LPS or aCD3/aCD28 stimuli. Samples were incubated for 48h at 37°C, supernatant was isolated and frozen. Such supernatants were tested for the presence of IFNg, TNFa, IL-1a, IL-1b, IL-4, IL-5, IL-8, IL-9, IL-10, IL-13 and IL-17 by means of a cytometric bead array (BioPlex-200, Bio-Rad Labs). Results: A total of 254 samples (47 healthy, 207 asthma) was processed. All groups were distributed equally among assay plates and there was no significant plate effect on the level of cytokines for any subject group. IFNg, TNFa, IL-1a and IL-1b were lower in LPS-stimulated samples of asthmatics and levels were inversely proportional to asthma severity. IL-1a, IL-1b and IL-9 in aCD3/aCD28-stimulated samples of asthmatics significantly and directly correlated to percentage of eosinophils in blood. IL-1a in LPS-stimulated samples were inversely associated with the percentage of eosinophils in the sputum. Nearly all cytokines measured following either stimulation were inversely correlated with the number and percentage of blood neutrophils. No correlation between cytokine levels & sputum neutrophils, FeNO, tIgE, or age of onset was observed.

Conclusions: Our findings regarding the levels of IL-1 in stimulated blood samples are novel and are worth exploring further for future treatment approaches. Our next steps include correlation with the presence of atopy as well as a data-driven, unsupervised approach for identifying disease endotypes.
Abstract No. 030

Comparison of the analytical performance of the Euroline®, ImmunoCAP®, and ImmunoCAP ISAC 112® in vitro allergen sIgE diagnostic assays in the pediatric arm of the DZL All Age Asthma Cohort (ALLIANCE)

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Background: Allergen extract-based versus (vs.) molecular allergen component-based sIgE determination assays vary in their diagnostic performance. We aimed at comparing conventional with component-resolved allergy diagnostics in children recruited at BREATH.

Methods: The Euroline® (EL) (Euroimmun) immunoblot (37 extracts and components), the ImmunoCAP ISAC 112® (ISAC) (ThermoFisher Scientific, TFS) microarray (112 components) and the ImmunoCAP® (CAP) (TFS) sx1 (8 extracts) and fx5 (6 extracts) panels were tested among 97 children (69 asthmatic and 28 healthy). A total of 14 allergens could be compared across assays.

Results: Prevalence of allergen sensitization (threshold ≥ 0.7 kU/l) was higher based on the CAP assay for all allergens tested except for peanut (more positive cases detected by ISAC) and soy (equal number of positive cases detected by EL). Lowest detection rates were associated with EL for all allergens compared. Overall (and HDM) allergen sensitization concordance vs. the CAP demonstrated higher sensitivity of the ISAC and comparable specificity between ISAC and EL. Quantitative (including CAP class) concordance between CAP and EL depicted a higher analytical sensitivity for CAP but also discrepant results (eg high HDM sIgE with CAP and negative with EL). Allergen extracts with CAP or EL were also compared vs. individual allergen components as assessed by ISAC. For both aero- and food-allergens there were samples which were positive for a molecular component although negative for the respective extract. Such cases were more abundant when the comparison was vs. EL as compared to CAP.

Conclusions: We observed discrepancies in detection rates of allergen sensitization between the applied methods. Such differences are important for atopy screening and monitoring especially in large epidemiological studies. Our next steps include qualitative concordance for each allergen, comparing sIgE against allergen extracts vs. the sum of components as well as evaluation of the diagnostic performance of the index tools.
Abstract No. 031

**Studies of the influence of asthma-associated cytokines on dendritic cell interaction with the airway epithelium.**

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Asthma is an T-lymphocyte controlled disease characterized by inflammation, mucus hyperproduction and airway remodeling. The airway epithelium has importance as physical barrier to the environment and is the first contact site for airborne substances such as microbes or allergens. Therefore, it is considered to be essential in regulating an asthmatic inflammation. It was shown that T cell-derived cytokines associated with ongoing asthma imprint well defined gene expression patterns in primary epithelium. Dendritic cells (DCs) are central players in asthmatic inflammation by bridging the innate and adaptive immune system and signals derived from the epithelium can highly influence their activation mode. Our first object was to study the interaction of DCs with airway epithelium in a 3D co-culture model when imprinted by asthma-associated cytokines and challenged with house dust mite extract of *Dermatophagoides pteronyssinus* (HDM). First, microarray analysis was performed on the airway epithelium cell line Calu-3 cultured under air-liquid interface (ALI) or submerged conditions in presence of the Th2 cytokines IL-4, IL-13 or the Th1 cytokine IFN-γ to characterize the differences in the gene expression patterns. Further, we stimulated 3D ALI co-cultures of Calu-3 cells and DCs and found marked differences in mRNA induction and cytokine release when Calu-3 ALIs were cytokine imprinted. Since several studies showed that bacterial cowshed isolates are asthma-protective in mouse models of acute allergic inflammation our second object was to study the influence of such a bacterium, namely *Lactococcus lactis* G121 (*L. lactis* G121), on the outcome of the immune reaction in 3D ALI co-cultures. Preincubation with *L. lactis* G121 at the time of imprinting indeed resulted in a modulated immune response evoked by HDM stimulation. In conclusion, our results support the sustained influence of T-lymphocyte-derived cytokines on airway epithelium and reveals the complexity and importance of the cellular interplay in DC/airway epithelium co-cultures.
Abstract No. 032

Dysregulation of epithelial NF-κB signalling leads to airway remodelling processes in Drosophila melanogaster

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Despite Asthma being one of the most abundant chronic diseases, the molecular framework underlying especially its severe forms is mostly not understood. We use Drosophila melanogaster as a model to investigate the effects of NF-κB dysregulation on the structural integrity and gene expression of the airway epithelium. NF-κB factors are not only central transducers of innate immune responses, they also act as important molecular integration points that determine cell fate. NF-κB can induce a cell survival program, accompanied by the release of anti-microbial peptides (AMPs) or initiating a cell death program by activating the JNK pathway. Dysregulation of this pathway was achieved by ectopic expression of different components of the innate immune signalling pathway that converges onto NF-κB activation. Moreover, we analysed the effects of the A20 homolog trabid that acts as an inhibitor of NF-κB signalling. These various manipulations consistently led to an increase in epithelial sizes as well as to hyper- and metaplasia. Expression analysis by quantitative real-time PCR of trabid-KO and rel-68 under normoxic and hypoxic conditions revealed a priming effect of the epithelial innate immune system. Meaning that animals that experienced this type of manipulation expressed more inflammatory mediators under basal and hypoxic conditions. Especially, the A20 homolog knockout showed and significant increase in upd2 (Leptin homolog) expression. Furthermore, under hypoxic conditions upd2, upd3 (IL-6 homolog) and AMPs were significantly increased. A modified version of this transgenic system is currently used to study compounds that rescue the NF-κB induced structural changes in a high throughput format.
Abstract No. 033

Ex-vivo RSV infection and inflammatory response in human and non-human primate precision-cut lung slices (PCLS)

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Respiratory Syncytial virus (RSV) is the most common pathogen infecting lower airways in children and there is increased evidence for higher risk of subsequent asthma. RSV infection is characterized by the cytopathic effect of syncytial formation and triggers the innate immune response to release anti-viral cytokines and chemokines, e.g., IP-10. In this work, we hypothesized that RSV could infect precision-cut lung slices (PCLS) from humans and non-human primates (NHP), producing an anti-viral immune response. Lungs from human donors and non-human primates (Rhesus macaques, Macaca mulatta) were used to prepare PCLS containing airways. The lung slices were inoculated with human-RSV-A2, UV-inactivated RSV, or medium from 1 up to 5 days. Supernatants, lysates, or slices were collected for viral load, tissue viability, and immune response assays. The inocula infectivity of 10⁶ and 10⁵ IU/ml was confirmed by plaque assay on Hep-2 cells, producing the typical cytopathic effect with syncytial formation. Infected cells were detected by immunofluorescence staining using an anti-RSV FITC-labeled monoclonal antibody. Live/DEAD staining and LDH assay showed a slight decrease in tissue vitality after RSV infection. Immune response was assessed by human-specific enzyme-linked immunosorbent assays (ELISA). RSV 10⁵ IU/ml significantly increased the release of the anti-viral chemokine IP-10 in NHP-PCLS, reaching a 4-fold increase at day 1 and 3-fold increase at day 4. At day 5, the increase was not significant. In human-PCLS, 24h incubation after RSV infection provoked a 90-fold increased IP-10 release, representing 15 times more response than in macaque-PCLS. PCLS can be used to study RSV infection ex vivo. Although non-human primates are not a natural host for RSV, lung slices provided from rhesus macaque could be infected, presenting anti-viral immune response comparable to human PCLS. In the future, these systems can be used to further investigate RSV mechanisms, especially in the context of asthma development.
Abstract No. 034

The establishment of human and murine Air-Liquid Interface (ALI) cultures for investigations of chronic inflammatory lung diseases at the ARCN Primary Cell Culture Lab

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The airway epithelium is the first line of defense, protecting the lung from inhaled pollutants, allergens and microbes. Therefore, the airway epithelium is equipped with ciliated cells and covered by a thin layer of mucus to prevent passage of foreign particles into the body. Beside acting as a physical barrier, airway epithelial cells express various pattern recognition receptors, such as Toll-like receptors in order to fight bacteria and viruses. Impairment of epithelial integrity by injury or inappropriate repair processes can support the development of chronic inflammatory lung diseases. Thus, it is of critical importance to understand the role of the airway epithelium in disease development and progression.

It is the aim of the ARCN Primary Cell Culture Lab to provide DZL researchers from various disease areas a platform to be able addressing selected research questions in the human and murine ALI culture model.

Mouse tracheas were used for the isolation of tracheal epithelial cells (mTEC). Human bronchial airway epithelial (hBEC) cells were isolated from main bronchi. In addition, hBECs obtained from Lonza were used. Both, hBECs and mTECs were seeded on transwell filters. After reaching confluency, the apical medium was removed to establish an ALI condition. Gene expression and cell morphology was assessed at various time points.

The ALI culture of hBEC and mTEC for 28 and 11 days, respectively, resulted in a pseudostratified epithelium, consisting of basal, club, goblet and ciliated cells. Gene expression analysis showed time-dependent increase of cell markers for ciliated (FoxJ1), club (CC10) and goblet cells (Muc5AC), whereas the basal cell marker CK5 decreased during differentiation. This was also confirmed in immunohistochemical stainings.

In conclusion, primary human and murine ALI cultures showing these typical airway characteristics are a powerful method to investigate various questions related to the role of the airway epithelium in health and disease.
Abstract No. 035

Smoking in early developmental windows selectively affects male flies

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Background Prenatal cigarette smoke exposure (CSE) is a major risk factor of developing childhood asthma. Furthermore, the likelihood of developing asthma may be propagated across generations. So far, *Drosophila* has been successfully used to explore mechanisms of epithelial immune dysfunctions and airway remodeling, which both are hallmarks of asthma. Being characterized by short generation time, high fertility and high percentage of identity and similarity to human genes, the fly emerges as a useful model to study conserved mechanisms of transgenerational inheritance.

Aim We aim at establishing a transgenerational *Drosophila* smoking model to identify highly conserved mechanisms modulated by CSE.

Methods Larvae were exposed to mainstream CS (4 puffs/minute) generated by a standard smoking machine (SCIREQ, Emka). The Cyp18A1 expression (Cyp1A1 homologue involved in xenobiotic metabolism) was measured both in whole larvae and isolated airways by qPCR. To assess the airway epithelial transcriptome, dissected airways derived from CS-exposed larvae were analyzed using RNA-sequencing. Larval viability was determined by counting numbers of living larvae, pupae, and emerging adults. Survival of the latter was assessed for 74 days.

Results The expression level of Cyp18A1 was significantly increased in whole larvae as well as dissected airways after CSE. Transcriptome analysis revealed that genes predominantly involved in xenobiotic metabolism and oxidative stress response were strongly upregulated in response to CSE. Most strikingly, signaling pathways mediating airway development and repair were primarily affected. While CSE during the larval stage did not affect the survival of emerging adults, the mortality rate of male but not female larvae was significantly enhanced.

Conclusion We established a juvenile *Drosophila* smoking model and observed a sex difference in sensitivity to CSE. In transgenerational studies, we will investigate if conserved mechanisms are involved in mediating CS-induced alterations in airway epithelial cells.
Abstract No. 036

Time-resolved eicosanoid profiling in stimulated human blood cultures from adult asthmatic subjects in comparison with healthy controls

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Background: Asthma is an obstructive chronic inflammatory disease, caused by overreaction of the airways in response to various stimuli. Eicosanoids, derived from polyunsaturated fatty acids contribute to the inflammatory process and some specific metabolites have been shown to play a key role in asthma development and acute exacerbations. However, a detailed understanding of the complex regulation of whole eicosanoid biosynthesis pathways in asthmatic subjects is still missing.

Objectives: Comprehensive analysis of eicosanoid profiles from stimulated blood cultures of asthmatic subjects compared to healthy controls.

Methods: Blood was collected from 173 asthmatic adults and 33 healthy controls and after stimulation with zymosan for either 4 or 48 h, eicosanoids were extracted and analyzed using a targeted LC/MS² approach.

Results: We found significant differences with distinct time kinetics in the activity of the five main eicosanoid biosynthesis pathways. After 4 h of stimulation metabolites belonging to the 5-Lipoxygenase (5-LOX) pathway show increased median levels within the group of asthmatic patients. In contrast, metabolites belonging to the cyclooxygenase (COX), 12- or 15-LOX and to the cytochrome P450 monoxygenase (CYP) pathway were produced at lower concentrations compared to the healthy controls. An opposite pattern was observed after prolonged stimulation. Whereas the concentration of most metabolites did not change between 4 h and 48 h of stimulation within the control group, we observed a decrease in the amount of eicosanoids of the 5-LOX pathway in the asthmatics after 48 h of stimulation. In contrast, these patients exhibit an increased production of metabolites belonging to the COX, 12- and 15-LOX pathway. Metabolites of the CYP pathway tend to increase after 48 hours of stimulation, reaching a median level comparable to those of the control group.

Conclusion: Asthmatic adults show a time-dependent distinct pattern of eicosanoid production which may contribute to the pathogenesis of chronic airway inflammation.
Abstract No. 037

**Comparison of Upper and Lower Airway Biomarkers Diagnosing Allergic Asthma**

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**RATIONALE:** Upper and lower airways share air transport, physical barrier, mucociliary clearance and immune interface as common features. Therefore, nasal secretions of the upper airways could represent an easy and non-invasively accessible proxy mirroring lower airway inflammation.

**METHODS:** Sixteen healthy participants and 15 non-smoking patients with mild asthma were recruited. In addition, clinical scores (rhinoconjunctivitis questionnaire on life quality (RQLQ), asthma control score (GINA), and psychosis screening questionnaire (PSQ)) were assessed. Sputum supernatants as well as nasal secretions were analyzed by electrochemiluminescence multi-array technology. To identify potential proxy biomarkers, we analyzed the strength of interaction between signals of upper and lower airways using a two-sided Spearman's rank correlation.

**RESULTS:** Correlation of one cytokine with one of the other mediators was frequently observed in the intra-organ comparison such as pro-eosinophilic cytokine IL-5 correlated with CCL-26. A strong correlation was also observed for nasal CCL-26 and nasal IL-13 (r= 0.57, p < 0.0001). Further, the correlation of sputum IL-13 and sputum CD19+ B cells (r= 0.36, p= 0.03) could indicate an IL-13-promoted production of IgE and IgG4 in B cells. This is supported by the finding that B cells of asthmatic patients showed a significant induction during grass pollen season compared to off season (p= 0.036). Periostin was correlated negatively with other mediators including itself when compared with upper airways. Furthermore, periostin negatively correlated with CD25+CD127- Tregs (r= - 0.65, p < 0.0001) and sputum eosinophils (r= 0.36, p < 0.05). In addition, the RQLQ seemed to be associated to levels of lower airway periostin (r= 0.38, p= 0.021).

**CONCLUSION:** Nasal proxy-biomarkers seem to be present for type-2 cytokines as IL-24. Periostin correlates with clinical symptoms but could be of limited use as proxy-biomarker, due to opposing correlations between proteins of upper and lower airways.
Abstract No. 038

Cytokine Patterns of the Upper Airways Distinguishing Several Sub-phenotypes of Pediatric Asthma

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RATIONALE: Childhood onset asthma is an umbrella term for various disease entities, which may differ with respect to pathomechanisms, cell subsets, and cytokine signatures. Thus, measurement of cytokines in nasal secretions could be a non-invasive method for molecular phenotyping of pediatric asthma.

METHODS: In nasal secretions of 332 children, IL-1α, IL-4, IL-5, IL-10, IL-13, IL-17, IL-22, IL-24, IL-33, IL-37, IFN-γ, IFN-λ3, CCL-20, CCL-26, G-CSF, periostin, SCGB1A1, TNF-α, TSLP were measured by electrochemiluminescence technology. Cytokine patterns were explored by factor analysis separately for children ≤6 years (n=121), >6 years (n=112) and for mono-/multitrigger wheezers (n=230) and related to wheeze or asthma phenotypes defined by a latent class analysis including symptoms and disease control parameters. Cytokine patterns were related to atopy as determined by specific IgE≥0.7 kU/L, FeNO and lung function.

RESULTS: The stratification by measured atopy for children >6 years revealed differences in FENO levels and one significantly distinguishing cytokine factor, representing higher levels of Periostin in atopic children. In children ≤6 years, the almost completely as non-atopic identified “severe low-controlled” phenotype differed in two cytokine profiles significantly to two controllable (one highly atopic, one rather non-atopic) phenotypes. One cytokine profile represented trendwise higher levels of IL-1α, IL-4, IL-13, IL-17, IL-22, and IL-37, while the other profile represented lower levels of IFN-λ3 and secretoglobulin1A1.

Stratification by IgE-levels for children >6 years revealed one factor distinguishing these, representing significantly higher levels of CCL-26, IL-5 and periostin, additionally for IFN-λ3 and Secretoglobulin1A1. Population stratification into phenotypes of mono-/multitrigger wheezers, the latter presented significantly decreased levels of G-CSF, CCL-20, IL-8, IL-17 and TNF-α, IL-13, IL-4, IFN-γ, and by trend increased levels of IFN-λ3 and secretoglobulin1A1.

CONCLUSION: Non-invasive measurement of nasal secretion cytokines in children appears to be feasible to analyze secreted protein levels. The panel of cytokines measured in the ALLIANCE children cohort seems to be suitable of distinguishing several sub-phenotypes of pediatric asthma.
pediatric asthma and wheeze. Nasal secretions therefore might be a valuable proxy of disease processes in the airways and complement inflammatory phenotyping of asthma.
Disease Area Chronic Obstructive Pulmonary Disease (COPD): Abstract No. 101 – 122
Abstract No. 101

**Alpha1-antitrypsin inhibits the ATP-induced release of monocytic interleukin-1β independent of its anti-elastase activity**

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**Introduction:** In addition to its well-described anti-protease activity, alpha1-antitrypsin (AAT) exerts anti-inflammatory functions. Interleukin-1β (IL-1β) is a pro-inflammatory cytokine of innate immunity. Increased IL-1β levels in patients after cardiopulmonary bypass (CPB) surgery are associated with the development of systemic inflammatory response syndrome. The anti-protease activity of AAT is diminished during CPB surgery as a result of oxidation and protease-complex formation. We recently demonstrated that AAT inhibits ATP-induced IL-1β release from human monocytes. Here, we tested different AAT preparations in order to correlate anti-inflammatory and anti-protease functions of AAT.

**Materials and Methods:** Native AAT from the plasma of healthy persons and from CPB patients was purified by affinity chromatography. N-chlorosuccinimide (NCS) was used to perform protein oxidation to inactivate the anti-elastase function of AAT. Human monocytic U937 cells were primed with lipopolysaccharide and subsequently stimulated with BzATP for 30 minutes in the presence or absence of AAT. The concentration of IL-1β in cell culture supernatants was measured by ELISA.

**Results:** We demonstrated that physiological concentrations of native, purified AAT from healthy donors, CPB patients and the AAT preparation Prolastin® dose-dependently inhibit the BzATP-induced IL-1β release in U937 cells. Upon oxidation, the inhibitory activity of AAT is unimpaired, whereas Respreeza®, a commercial AAT preparation that is chemically reduced during purification, is inactive. Interestingly, oxidation of Respreeza® reactivates its anti-inflammatory function. Furthermore, within the first 15 minutes of CPB surgery, no inactivation of the anti-inflammatory potential of AAT could be observed.

**Conclusions:** Native AAT from healthy donors and the AAT preparation Prolastin® efficiently inhibit the BzATP-induced IL-1β release from monocytic cells. This effect is independent of the anti-elastase activity of AAT. Furthermore, the capacity of AAT to inhibit IL-1β release seems to be unimpaired within the first 15 minutes of CPB surgery. However, later time-points of CPB surgery need to be investigated.
Abstract No. 102

**Comparative transcriptomic analysis reveals differences in PBMCs from COPD and CAP patients**

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Chronic obstructive pulmonary disease (COPD) is a poorly reversible condition that is characterized by airflow limitation and decrease of lung function. In 2012 COPD-related deaths amounted to more than 3 million, which accounts for 6% of the worldwide deaths in 2012. COPD is estimated to become the 3rd major cause of death by 2020. Community acquired pneumonia (CAP) is clinically defined by a sudden onset of severe illness that is accompanied by signs of lower respiratory tract infection, fever, cough and dyspnoea. While subject to variance due to region, season and population characteristics, the incidence of CAP is estimated to lie between 1.5 and 14 cases per 1,000 persons per year, children under 5 years of age and the elderly of more than 65 years being most strongly affected. We investigated the PBMC transcriptome of 5 healthy donors, 6 pneumonia patients and 6 COPD patients with acute exacerbations. Hereby, we identified regulatory networks, consisting of microRNAs and mRNAs that converge on Inflammation of the Lung and Interleukin 6. While the CAP and COPD networks are similar in nature, the COPD exacerbation signature seems to be more complex, as it contains a broader core network, featuring exclusive factors such as ANG, CD163 and FLT3, and also bystander node interactions, such as LEF1/TLE2. Both of these factors are associated with repression of Wnt signaling, which is inhibited in COPD. Factors that seem to be specific to CAP include the transcription factor E2F1 and CYP1B1 (cytochrome P450 subclass).

In summary, we gathered a broad and conclusive transcriptional map of PBMCs from CAP and COPD patients in comparison to healthy donors. The described factors may yet serve as accessible markers of disease in peripheral blood.
Abstract No. 103

**Identifying predictors of healthcare utilization and costs in COPD patients over 18 months: first longitudinal results of the German COSYCONET COPD cohort**

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**Background**

COPD is associated with excess healthcare utilization and represents high costs to health systems. With regard to time trends, this study reports the first longitudinal results on healthcare utilization and associated costs from the German COSYCONET prospective cohort study.

**Methods**

We analysed data from 1907 COPD patients participating in the German COSYCONET cohort study with available information on baseline and 18-month follow-up visit. Besides descriptive comparison of disease status and healthcare utilization at the two time points, generalized linear regression models were used to quantify the extent to which variables measured at baseline predict future direct costs at the 18-month follow-up. A generalized estimating equation model was used to obtain adjusted mean direct costs at baseline and follow-up. All models were adjusted for GOLD grade, age, sex, education, BMI, smoking status, comorbidity count, years since COPD diagnosis, symptoms, and exacerbation history.

**Results**

Overall, we found a significant decrease of lung function (FEV1 (liter) 1.7 vs. 1.6) with a corresponding increase of the proportion of participants perceiving dyspnoea during walking (mMRC >2, (14.5 (%) vs. 18.1), both indicating a deterioration of condition over time. No significant increase in overall costs was observed in 18 months, but higher baseline COPD grades were significantly associated with higher total direct costs and inpatient and medication costs at the follow-up visit. A history of severe exacerbations in the previous 12 months and comorbidity count >3 at baseline were also found to be significant drivers of total direct costs. Results of the GEE model reveal that changes in adjusted mean direct costs between baseline and follow-up were not significant across all COPD groups.

**Conclusions**

Our findings underline the importance of managing symptoms, exacerbations and comorbidities in the treatment of COPD patients in order to control the development of direct healthcare costs of COPD over time.
Abstract No. 104

Validation of Cell Analysis by Chipcytometry in Induced Sputum Cells

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Introduction: Chipcytometry combines the phenotyping ability of flow cytometry with detailed imaging and functional insight of microscopic technologies. Immobilized cells on microfluidic chips can be stored for several months and multiple cellular markers can be iteratively analyzed. The aim of the present work is the validation of cell analysis by Chipcytometry for induced sputum. Induced sputum is a complex matrix possessing a broad spectrum of cell morphologies and contamination with airway epithelial cells. Further, homogenization with reducing agents is needed. Guideline-compliant validation of cell analysis with surface marker antibodies is a prerequisite for acceptance of the use of this methodology in clinical trials. Methods: Selected cell surface markers were validated (CD3, CD4, CD8, CD14, CD16b, CD45). Sputum samples of at least 4 subjects (healthy, COPD, and asthma) were processed, pooled, loaded onto chips and iteratively stained using the respective antibodies followed by bleaching cycles in triplicates. Variances in antibody staining were investigated. The immune status was obtained and compared with gold standard methodology flow cytometry and standard light microscopy. Results: Lymphocytes, neutrophils, monocytes and macrophages were identified by combining cell surface markers and morphological characteristics (cell size and nuclear staining). Cell differentiation by Chipcytometry using surface marker expression and cell morphology was similar to data obtained using flow cytometry and light microscopy. Lymphocyte and neutrophil numbers showed comparable percentages between all methodologies. CD14 expression and macrophage countings were reduced in Chipcytometry. Conclusion: Measurement of CD3, CD4, CD8, CD16b and CD45 by Chipcytometry showed reliable and reproducible results on sputum cells. Data was comparable with flow cytometry and light microscopy. Chipcytometry represents a feasible tool for assessment of the immune status. Improved sputum preparation methodology need to be developed to avoid cell plaque formation on chips, which disturbs automatic identification of macrophages, by the cell recognition software.
Abstract No. 105

**Drosophila melanogaster as a model to study effects of maternal e-nicotine exposure on offspring**

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**Background:** Prenatal exposure to nicotine due to maternal smoking in pregnancy can have lifelong effects on the offspring respiratory system leading to increased risks of asthma and COPD. Moreover, pregnant women have started to use e-cigarettes at the rates at least equal to conventional cigarettes; however, little is known how e-cigarettes affect the offspring’s respiratory health. So far, mouse models are extensively used to investigate the effects of inter- and transgenerational maternal nicotine exposure. However, these models are burdened by long generation times and high breeding costs. Therefore, we chose *Drosophila melanogaster* for our intergenerational nicotine-exposure studies due to its high fecundity, short generation time and the fact that its tracheal system shares common features in development, physiology and function with the mammalian airways.

**Aim:** To establish a *Drosophila melanogaster* model for studying intergenerational effects of maternal e-nicotine-exposure on airway development.

**Methods:** Virgin female *Drosophila* flies were exposed to 5mM of nicotine vapor for ten seconds every hour for a total of eight times (control group: water vapor) using a self-designed nicotine-laced vapor apparatus. Following the last exposure, e-nicotine and sham exposed female flies were mated with male flies of the same age. The F1 generation was then analyzed for viability, size and weight of larvae, viability of pupae, developmental time, weight and size of hatched flies.

**Results:** We observed relative developmental delays during larval and pupal stages in the F1 generation. In addition to this, the size and the weight of first instar larvae were decreased compared to the control group. Other parameters remained unchanged.

**Summary:** As maternal nicotine-exposure influences growth and development of the F1 generation, we propose that our established model could be useful for studying molecular mechanisms and signaling pathways mediating intergenerational changes of e-nicotine exposure.
Abstract No. 106

The role of mitochondria in biological aging of the lung

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Introduction: During aging, the lung undergoes structural and functional changes associated with a decrease in lung function. It has been suggested that mitochondrial dysfunction may play a crucial role in this process. Different proteins are implicated in mitochondrial ROS metabolism and apoptosis, including the adaptor protein p66shc and cyclophilin D (CypD). Thus, we investigated the role of p66shc and CypD in aging of the lung.

Results: The mean linear intercept (MLI) and the degree of muscularization of pulmonary arteries (DMPA) in wild type (WT) mice were not altered during aging. In contrast, the MLI in 24 months old and the DMPA in 12 and 24 months old p66shc−/− mice were increased compared to WT mice of the respective ages. 24 month old CypD−/− mice showed an increased MLI. The expression of ER stress marker, XBP1 was higher in the lungs of p66shc−/− mice as well as in CypD−/− mice during aging.

Conclusion: The mitochondrial proteins p66shc and CypD may play an important role during aging of the lung. The deletion of both proteins CypD and p66shc could promote the development of emphysema via increasing ER stress.
Abstract No. 107

Survival after endoscopic valve therapy in patients with severe emphysema

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Background: Endoscopic valve therapy leads to an improvement of lung function, exercise tolerance and quality of life in a selected cohort of patients with advanced emphysema. So far, only few data exist on the long-term outcome. This analysis evaluated the impact of valve therapy on the survival of emphysema patients.

Methods: Survival rates of patients with advanced emphysema who underwent endoscopic valve therapy were assessed according to their radiological outcome following valve placement. Baseline of survival was set as the date of first valve implantation.

Main findings: From 2005-2013, 449 patients (mean age 64±7 years, 54% male) with emphysema underwent endoscopic valve therapy and were followed for a mean time of 37.3±21.3 months. 128 patients (29%) developed complete lobar atelectasis, 34 out of these also experienced a pneumothorax; in 50 patients (11%), pneumothorax without lobar atelectasis and in 261 (58%) patients only target lobe volume reduction or no volume change were observed. Patients with atelectasis showed significantly better baseline FEV1%, RV(l), TLC(l) and TLCO (%) (all p <0.05); however, the difference was not clinically relevant (FEV1 32±8% vs. 30±9%, RV 5.4±1.2L vs. 5.8±1.4L, TLC 7.9±1.6L vs. 8.2±1.7L, TLCO 32±12% vs. 30±11%). Patients with lobar atelectasis after valve placement had a significant survival benefit compared to patients without atelectasis (p=0.009; 5-year survival rate 65.3% vs. 43.9%). The advent of pneumothorax in 84 patients did not influence survival (p=0.52).

Conclusions: Lobar atelectasis following endoscopic valve therapy is associated with a survival benefit.
Abstract No. 108

**Epigenetic regulation by arginine methylation controls monocyte migration and COPD pathogenesis**

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Chronic obstructive pulmonary disease (COPD) is an inflammatory lung disease characterized by progressive airflow limitation. Cigarette smoke is a major risk factor for the development of the disease, but currently a role for epigenetics in the underlying pathology of COPD is emerging.

Histone arginine methylation by protein arginine methyl transferases (PRMTs) is one of the most important epigenetic controls that regulate gene expression. PRMTs are involved in a number of biological processes including signal transduction, cancer tumorigenesis, transcriptional control, and DNA repair. PRMTs are associated with the regulation of important pathways like Wnt signaling and epithelial to mesenchymal transition which is linked to COPD pathogenesis.

PRMTs expression is increased in COPD patients and immunohistochemistry staining demonstrated that PRMTs are mostly localized in macrophages. Moreover, macrophages which were isolated from cigarette smoke exposed mice expressed increased Prmt7. To understand the mechanism, micro-array analysis of bone marrow derived macrophages from wild-type and Prmt7 reduced mice was undertaken and Ingenuity Pathway Analysis suggests disrupted leukocyte extravasation signaling. To support these findings, CRISPR/Cas9 generated stable knock down of Prmt7 in the MHS cell line is used and confirmed loss of Prmt7 results in impaired macrophage adherence and migration.

In summary, we demonstrate that PRMT regulated histone epigenetic marks control macrophage recruitment to the lungs and subsequent COPD pathogenesis.
Abstract No. 109

**Impaired FGF10 signalling in the lung leads to development of COPD and pulmonary hypertension**

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Chronic obstructive pulmonary disease (COPD) is a global health problem, which is characterized by persistent airflow limitation and alveolar septal wall destruction resulting in emphysema. Additionally, COPD patients often suffer from pulmonary hypertension (PH) due to significant structural remodelling of the vasculature. Among other molecular mechanisms, influx of inflammatory cells, as well as misbalance of apoptotic and proliferating cells, are suggested to be important in the pathology of COPD.

In our experiments, as an animal model of COPD and PH, we investigate mice which are chronically exposed to cigarette smoke. Our preliminary results showed alteration of fibroblast growth factor 10 (FGF10) in lungs from cigarette smoke-exposed mice. Therefore, we aimed to investigate the importance of FGF10 signalling in COPD and PH using FGF10 and FGF receptor 2b (FGFR2b) haploinsufficient mice.

Lung function and hemodynamic measurements show that our transgenic mice develop emphysema and PH spontaneously with aging. FGF10 and FGFR2b haploinsufficient mice are more prone to develop other COPD features such as excessive inflammation and high nitrotyrosine formation in the lung. Furthermore, these animals are more susceptible to cigarette smoke-induced damage. Our results suggest cigarette smoke could impair FGF10 signalling in the lung, leading to the development of COPD and PH.

Supported by the DFG, WE1978/8-1
Abstract No. 110

The revised GOLD 2017 COPD categorization

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Introduction

The COPD classification proposed by the Global Initiative for Obstructive Lung Disease was recently revised, and the A to D grouping is now based on symptoms and exacerbations only. Potential associations with comorbidities have not been assessed so far. Thus the aim of the present study was to determine the relationship between the revised (2017) GOLD groups A-D and major comorbidities.

Methods

We used baseline data from the COPD cohort COSYCONET. Comorbidities were identified from patient self-reports and disease-specific medication: gastrointestinal disorders, asthma, sleep apnea, hyperuricemia, hyperlipidemia, diabetes, osteoporosis, mental disorders, heart failure, hypertension, coronary artery disease. The A-D groups were based on either the COPD Assessment Test or the modified Medical Research Council scale. Exacerbations were also categorized as per GOLD recommendations.

Results

Data from 2228 patients were analyzed. Using GOLD group A as a reference, group D was associated with nearly all comorbidities, followed by group B and C. When groups A-D were dichotomized as AC vs. BD (symptoms) and AB vs. CD (exacerbations), all comorbidities correlated with symptoms and/or exacerbations. This was true for both mMRC- and CAT-based categorizations.

Conclusions

These findings suggest that the recently modified GOLD categorization is clinically relevant beyond being purely an assessment of symptoms and exacerbations. As the A-D groups correlated with the risk of important comorbidities, with some differences in terms of the correlation with symptoms and exacerbations, the findings underline the importance of identifying comorbidities in COPD, particularly in non-responders to therapy who have high symptoms and/or exacerbation rates.
Abstract No. 111

Uric acid, creatinine, lung function, physical capacity and exacerbation frequency in patients with COPD: a multi-dimensional approach

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Background
Recent investigations showed an increased mortality in COPD patients with elevated levels of uric acid, as well as associations with functional parameters and exacerbations. The aim of this study was to provide an integrative view of the multiple relationships to uric acid in COPD.

Methods
We used baseline data from the German COPD cohort COSYCONET which were evaluated by standard multiple regression analyses as well as path analysis to describe the network of relations between parameters, particularly uric acid.

Results
Data from 1966 patients were analyzed. Uric acid was significantly associated with airflow limitation, reduced physical capacity and higher exacerbations, as well as creatinine and risk factors such as BMI and packyears. These associations remained significant after taking into account the other multiple relationships between variables, or the diagnosis of hyperuricemia, and/or the presence of hyperuricemia-specific medication which led to a dissociation between creatinine and uric acid levels.

Conclusion
Within the limits of a statistical approach, our results strongly suggest that uric acid is not only a useful biomarker in COPD but plays a causative role for relevant outcomes in this disease. Whether this role can be substantiated in targeted intervention trials should be investigated in future studies.
The relationship between body weight, body composition and COPD is not fully understood. We investigated the association between BMI, body fat percentage (BFP) and common diseases in COPD patients as compared to lung-healthy controls within the framework of the German COSYCONET study.

In the COSYCONET COPD cohort and its population-based reference cohort KORA, anthropometric measurements and spirometry were performed and BFP was determined by bioelectrical impedance analysis. Subjects from KORA without known COPD, asthma or chronic bronchitis and with FEV₁/FVC ≥ 0.7 were regarded as apparently lung-healthy. Common diseases were assessed using standardised questionnaires/interviews. BMI and BFP were compared between COPD patients and controls adjusting for sex and age and accounting for GOLD grade using general linear models, BFP was additionally adjusted for BMI. Furthermore, BFP was compared between subjects with vs. without common diseases accounting for GOLD grade.

2289 COPD patients and 1221 control subjects were included (age range 40-90y). In COPD patients vs. controls, mean BMI and the percentage of subjects with BMI > 30 kg/m² were lower (26.6 vs. 28.1 kg/m² and 22% vs. 29%, respectively;p<0.01 each) while the percentage of subjects with BMI < 21 kg/m² was higher (11% vs. 3%;p<0.01). With increasing COPD severity, BMI decreased from 28.1 kg/m² in controls to 24.3 kg/m² in GOLD4 patients (p<0.05) while BFP was 32.3% in controls and increased from 31.3% in GOLD1 to 33.0% in GOLD4 patients (p<0.05). Subjects with vs. without hypertension showed a trend towards increased BFP reaching significance in GOLD4 (33.8% vs. 32.0%;p<0.05), whereas for diabetes a heterogeneous pattern was observed.

The decrease in BMI and concomitant increase in BFP with increasing COPD severity indicates wasting. While overall differences in BFP were comparably small, a positive relationship between BFP and hypertension was observed in advanced COPD. Possible influences of medication and systemic inflammation will be targeted in future analyses.
65

Abstract No. 113

Myh10 deficiency leads to defective ECM remodeling and pulmonary disease

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Impaired alveolar formation and maintenance are features of many pulmonary diseases that are associated with significant morbidity and mortality. In a forward genetic screen for modulators of mouse lung development, we identified the non-muscle myosin II (NM II) heavy chain gene, Myh10, as a crucial regulator of alveolar development and homeostasis. Myh10 mutant pups exhibit cyanosis as well as respiratory distress, and die shortly after birth. Loss of Myh10 function leads to differentiation defects in alveolar epithelial and mesenchymal cells as well as lung morphogenetic defects. From omics analyses and follow up studies, we found decreased Thrombospondin (THBS) expression accompanied with increased matrix metalloproteinase (MMP) activity in both mutant lungs and cultured mutant fibroblasts, as well as disrupted extracellular matrix (ECM) remodeling. Consistent with MYH10 expression data, we find that Myh10 is required in lung mesenchymal, but not epithelial, cells to regulate ECM composition. Mesenchyme-specific Myh10 deletion at early postnatal and adult stages also resulted in alveolar simplification. Notably, MYH10 expression is down-regulated in the lung of emphysema patients, suggesting a conserved function for MYH10 in lung homeostasis. Altogether, our findings reveal critical roles for Myh10 in epithelial cell differentiation and alveologenesis at least in part via the regulation of ECM remodeling, which may contribute to the pathogenesis of emphysema.
Abstract No. 114

Development of an in vitro model to study the influence of cigarette smoke on the human bronchial epithelium

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**Background:** Being directly exposed to inhaled air, the respiratory epithelium is influenced by many environmental factors that can disrupt its barrier integrity and immune functions. Cigarette smoke (CS) is major risk factor for the development of chronic lung diseases (CLD), and thus plays an important role in both disease initiation and progression. Recently, also changes in the composition of the lung microbiota have been associated with CLD (Huang et al., 2010), but CS influence on the composition is insufficiently understood.

**Aim:** To study the interaction between airway epithelial cells and bacteria, and analyse how this is influenced by CS, we first aimed to establish an *in vitro* CS model.

**Methods:** Human bronchial epithelial cells (16HBE) were cultured at the air-liquid interface and exposed to mainstream CS generated from research cigarettes (3R4F, Kentucky, USA) or to fresh air using the P.R.I.T. ExpoCube system (Fraunhofer ITEM, Hannover, Germany). Particle concentrations were measured by light-scattering photometer. To establish ideal smoking conditions, the cells were exposed to lower (0.60 µg/cm³) or higher (2.84 µg/cm³) CS doses 1 hour/day for 3 days. After each exposure, transepithelial electrical resistance (TEER) measurement and cell viability assay (WST-1) were performed.

**Results:** The lower CS dose did not disrupt epithelial integrity or cause cell death in CS-exposed group. However, viability testing and TEER measurement showed that the higher dose was lethal and the cells died 48 hours after exposure, in contrast to the control and air-exposed groups.

**Conclusions:** Preliminary data showed that concentration of 0.60 µg/m³ allowed exposure without affecting cell viability. In next experiments, the cells will be exposed repetitively to same CS doses every day, to gather dose-dependency response. Together with data from mouse models, these results will help in further investigations of CS influence on the lung microbiota composition and its interaction with the bronchial epithelium.
Abstract No. 115

**Effects of lipopolysaccharide and alpha1-antitrypsin on IL-37 expression and release**

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Discovered in the year of 2000, relatively new member of IL-1 cytokine family, IL-37 is a potent anti-inflammatory cytokine. Studies have shown that this cytokine is a ligand for interleukin 18 receptor (IL18R1/IL-1Rrp) and mostly secreted by human blood monocytes. Out of all Toll-like receptor agonists, lipopolysaccharide (LPS) seems to be the strongest stimulus of IL-37 expression. However, the expression of IL-37 gene is relatively low in monocytes due to the short half-life of mRNA. In our study, we used adherent human peripheral blood mononuclear cell model and analyzed secretory levels and gene expression of IL-37. Cells were cultured for 4 hours alone and in the presence of different preparations of purified from human plasma alpha1-antitrypsin (AAT) and LPS (1μg/ml) separately or in combinations. Afterwards, cell supernatants were analyzed for the IL-37 release (ELISA). Cell lysates were used for IL-37 protein analysis (Dot blots and Western Blots) and mRNA was prepared for IL-37 expression (qRT-PCR). Our data show that LPS significantly induces IL-37 expression but has no effect on protein release. In combination with LPS, AAT markedly lowered LPS effect on IL-37 expression. In contrast, AAT alone showed only minor effect on IL-37 expression but induced IL-37 release. Unexpectedly, different preparations of purified AAT protein showed significant variability in the induction of IL-37 release. A series of additional experiments yielded that some of the AAT preparations react with anti-IL-37 antibody. This finding supports a concept that AAT may form a complex with IL37. Further research is ongoing to confirm or deny this observation.
Abstract No. 116

A novel demonstration of macrophage plasticity in an integrated human viable lung slice system

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Alveolar Macrophages (AM) are key drivers of innate immune responses to inhaled pathogens and particles. Within the past years it became apparent that diverse stimuli differentially drive AM polarization towards the classically activated (M1) or alternatively activated (M2) phenotype. AM polarization and function are thought to contribute to pathologies in respiratory disease indications, e.g. asthma, lung cancer, COPD and respiratory infections.

Aim of the study was to investigate if AM contained within their natural environment in fresh human lung tissue slices can be polarized by ex vivo stimulation.

Therefore, fresh human lung tissue slices were stimulated using either LPS/IFN-γ, IL-4 or IL-4/IL-13 for up to 3 days to polarize AM towards M1 or M2 phenotype, respectively. Viability was analyzed via lactate dehydrogenase release into tissue culture supernatant. Production of M1 or M2 associated cytokines was analyzed using ELISA. Localization of AM within the human lung tissue was visualized immunohistochemically.

Stimulation of AM in human lung tissue did not induce loss of viability after cultivation up to 3 days. Polarization of AM towards M1 phenotype was achieved using LPS/IFN-γ, as demonstrated by increased production of M1 related cytokines IL-6 and CCL2. Stimulation of human lung tissue with IL-4 or IL-4/IL-13 induced increased production of CCL17, indicating a polarization of AM towards M2 phenotype. Immunohistochemistry showed a colocalization of AM and cytokines indicating M1 or M2 polarization in viable lung tissue.

These results represent the first indication that polarization of AM in human fresh and viable lung tissue via classical and alternative activation can be achieved by ex vivo stimulation thereby mimicking pathways relevant in human lung disease. This enables us to further study the role of macrophage polarization in lung diseases such as asthma, COPD, lung cancer or respiratory infections using a more complex and integrated human viable lung tissue system.
Abstract No. 117

A Comprehensive Way to Rate Sputum Quality in Clinical Trials

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Analysis of induced sputum is widely used to assess airway inflammation. Quality of sputum is variable and currently defined by the level of squamous cells contamination (SQ%), which does not consider the quality of relevant sputum cells. Here we suggest a novel quality score and evaluated how it is related to differential cell count inter-observer variability and agreement.

Thirty sputum samples, with a broad range of quality, from three DZL sites were analyzed by nine evaluators. Slide quality based on cell morphology, the level of cellular debris and SQ% was rated on a 5-point scale (0, 0.5, 1, 1.5, 2; low-high) Inter-evaluator variability (SD), evaluator accuracy, intraclass-correlation coefficients (ICC) between evaluator and overall mean cell counts (as reference) were computed. To assess the relationship between these parameters and slide quality the dataset was split into quality levels A, B and C based on the mean slide score.

The overall mean (range) quality score was 0.9 (0.0; 1.6). Mean SQ% was 18.5 (1; 85) %. The correlation between slide quality and SQ% was significant for each evaluator, but with a wide range of r-values (-0.39; -0.69). The 17 slides from quality level A and B had a maximum SQ% of 22%, while 6 slides from the low quality level C had below 22% squamous cells. The SD for macrophages (AM) and neutrophils (NG) across evaluators was significantly decreased with increasing slide quality (p<0.02; p<0.01). The ICC for AM and NG was always >0.9 and increased for all evaluators, if low quality level C slides were excluded.

We propose to include sputum cell integrity, the amount of cellular debris and SQ% into a slide quality score. Excluding samples based on this score reduced differential cell count inter-evaluator variability and improved the agreement of evaluators with the overall mean differential cell count.
Abstract No. 118

Oltipraz reduces cigarette smoke induced lethality in a Drosophila COPD model

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COPD is one of the main leading causes of death worldwide and its incidence is rising steadily. The primary risk factor for the development of COPD is direct or indirect cigarette smoke (CS) exposure. We used Drosophila as a model to investigate the influence of CS exposure resembles the major characteristics of COPD. Chronic cigarette smoke exposure reduced life span substantially. Moreover, the animals show, exactly as human COPD patients, an increased metabolic rate and reduced body fat. Physical activities drop dramatically after prolonged exposure. Furthermore, we could show that a recurrent exposure to cigarette smoke leads to reduced respiratory surfaces in flies, which all together shows that chronic cigarette smoke exposure induces all major hallmarks of COPD in Drosophila. Inferred from RNA sequencing analyses, especially the Nrf2 (nuclear factor erythroid 2–related factor) and the JAK/STAT (Janus kinase/signal transducers and activators of transcription) pathways are highly upregulated in response to CS exposure. Among the regulated genes are especially those encoding for antioxidant mediators like members of the cytochrome P450 family, Glutathione S-transferases and Mucin-like proteins. Based on these results, we performed an intervention study with the Nrf2 activator Oltipraz. Treatment with Oltipraz induced Nrf2 signaling in the airway epithelium and increased lifespan of cigarette smoke treated animals significantly. Taken together, these results show the usefulness of this simple model for the development of novel treatment strategies for COPD.
Study of proximal bronchial epithelial cell (sub)populations in COPD

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The human airway epithelium protects the body from inhaled insults, such as smoke, allergens, or pathogens. Since it constitutes the first line of defense, its integrity and proper composition is crucial for healthy airway homeostasis. In COPD, the proximal airway epithelium is dramatically altered (Schamberger AC et al., 2014 & 2015) and manifests in chronic bronchitis. To date, no drug against clinical bronchitis exists since research mostly concentrates on emphysema and small airway disease, two other hallmarks of COPD.

The proximal airways are characterized by a pseudostratified epithelium, consisting of mainly ciliated, basal, and secretory cells (predominantly goblet and scare Clara cells). Basal cells possess stem cell capacity and can give rise to the other cell types (Rock et al., 2009). Mouse studies suggest that this process involves intermediate phenotypes and secretory cells as a major steady-state source for new ciliated cells (Rawlins EL et al., 2009, Tata PR et al., 2009, Watson JK et al., 2015), although this scientific concept has not been proven up to date.

Using immunofluorescence staining on freshly isolated primary human bronchial epithelial cells, on in vitro differentiated basal cells, or on FFPE tissue from healthy proximal airways, we were able to identify various intermediate epithelial cell types (p63/CC10 and CC10/MUC5AC double positives, MUC5AC/MUC5B double and single positives), demonstrating a higher degree of heterogeneity in the airway epithelium as previously known. These data point towards a differentiation route from basal over Clara to goblet cells and the existence of goblet cell subpopulations.

Single-cell analysis of proximal airway cells from healthy lung tissue will allow us to identify epithelial cell (sub)populations by the use of known and unknown markers and prove the proposed concept in more detail. Translation of these findings into the tissue and primary cells derived from COPD patients can unravel underlying disease mechanisms and characteristics aiming to identify treatable traits.
Abstract No. 120

Short-term effects of non-invasive ventilation during exercise in hypercapnic patients with very severe COPD – a randomized controlled cross-over trial

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Background There has been increasing interest in the use of non-invasive ventilation (NIV) during pulmonary rehabilitation (PR) as an additional tool to augment PR benefits in patients with advanced COPD. There are some hints that hypercapnic patients utilizing NIV during exercise might train for longer durations and/or at higher levels of exercise intensity.

Objective To investigate short-term effects of using NIV during exercise in hypercapnic patients with very severe COPD.

Methods Within a comprehensive 3-week inpatient PR program 20 hypercapnic (PCO2>50mmHg at rest and/or during exercise) COPD patients (GOLD IV) took part in this randomized-controlled cross-over trial. The day after an initial incremental cycle ergometer test, patients performed two constant work rate tests (CWRT) at 60% of the peak work rate, with and without NIV, in randomized order. Cycle endurance time (primary outcome), transcutaneous PCO2 (TcPCO2), oxygen saturation (SpO2) and perceived dyspnea/leg-fatigue (Borg scale) during CWRTs were investigated.

Results Participants (age 60±6yrs; FEV1 19±4%/pred.; PaCO2 51.0±6.8mmHg) performed CWRT at 26±11W. NIV via full face-mask was performed with IPAP/EPAP levels of 27±3/6±1cmH2O. During CWRTs patients cycled with NIV 663sec and without 476sec. This treatment effect of 187sec 95%CI[44 to 329] in favor of CWRT with NIV was significant (p=0.013). At isotime (=time of CWRT with shortest duration) TcPCO2 was significantly lower (-6.1mmHg 95%CI[-7.4 to -4.7] and SpO2 was significantly higher (3.6% 95%CI[1.4 to 5.7] with NIV. Furthermore, patients after CWRT with NIV perceived less dyspnea (p=0.008) with comparable leg-fatigue (p=0.79). For all outcomes no carryover effect was found (all p>0.05).

Conclusion We found that by using NIV during exercise hypercapnic patients with very severe COPD had a clinically relevant increase (>minimal important difference of 105s) in endurance time. NIV during exercise seems to be feasible and could be an opportunity to improve endurance training outcomes in very selected patients.
Abstract No. 121

Preclinical development of a biomarker signature predictive of COPD exacerbation

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**Background:** Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of death worldwide, its main risk factor being cigarette smoke (CS). Acute exacerbations in COPD are caused by viral infections resulting in progressive loss of lung function and increased mortality. The early prediction of imminent exacerbations would allow initiating therapy before symptoms are fully developed. This requires the development of predictive biomarkers that work both in moderate and severe COPD.

**Aim:** To develop a biomarker signature in a murine model of moderate COPD-like inflammation (“Test model”) and to validate this signature in a second COPD murine model with stronger inflammation and lung function deficits (“Validation model”).

**Methods** Female C57Bl6/J mice were exposed to CS (~6000 particles/m³) or room air daily for 24 days (Test model) or 56 days (Validation model). One hour after the last CS exposure, mice were treated intranasally with 0.1 µg, 1 µg or 10 µg poly(I:C) or with PBS alone. 24 hours later, immune cells and proteins were analyzed in BALF (bronchoalveolar lavage fluid) and lungs.

**Results** Test model: The percentage of different dendritic cells (DCs) was significantly elevated following poly(I:C) application in the CS-exposed group in comparison to controls (p<0.05). The CS-exposed mice receiving poly(I:C) showed high levels of IL-6, IL-1b and KC/GRO in BALF. Different protein patterns detected in a 2D-gel could be related to posttranslational modifications in the same group. The “Validation model” had a stronger inflammatory response and lung function deficits.

**Conclusion** The inflammatory response observed in CS-exposed mice receiving poly(I:C) differed significantly in comparison to the control groups. Since the cytokines that were increased in this model are also increased in patients with exacerbation, we suggest that our model could not only be used to reveal candidate biomarkers predicting exacerbations but further to understand the mechanisms underlying viral-induced COPD exacerbations.
Abstract No. 122

SLPI inhibits ATP-mediated release of IL-1β from human monocytes via secretion of a soluble factor

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Background: Mechanisms controlling IL-1β maturation are of big clinical interest, but so far remained largely unexplored. Our preliminary experiments provided evidence that secretory leukocyte protease inhibitor (SLPI), known as a major anti-protease of the lung, potently inhibits ATP-mediated release of IL-1β. In current studies we aimed to investigate the mechanism involved in the control of IL-1β release.

Methods: LPS-primed monocytic U937 cells were stimulated with BzATP, a P2X7 receptor agonist, in the presence and absence of SLPI. IL-1β released to the medium was monitored by ELISA. Pharmacological inhibitors and siRNA were used to investigate the pathway involved in the control of IL-1β release. In addition, to study the ion channel function of the P2X7 receptor in the presence of SLPI, two-electrode voltage-clamp measurements were performed on oocytes overexpressing the P2X7 receptor. To determine, whether the inhibitory mechanism mediated by SLPI involves the secretion of a soluble factor, LPS-primed U937 cells were stimulated with SLPI, cell-free conditioned medium was harvested after 30 min and fractionated by ultrafiltration. The low molecular weight fraction (< 3 kDa) was tested for its inhibitory effects on the BzATP-mediated IL-1β release.

Results: We demonstrated that SLPI inhibited ATP-mediated IL-1β release in a dose-dependent manner. SLPI did not directly modulate the ion channel function of the P2X7 receptor overexpressed in oocytes. Using a panel of inhibitors and siRNA, we identified the involvement of calcium-independent phospholipase A2β, Src kinase and nicotinic acetylcholine receptor sub-units α7, α9 and α10 in the SLPI-mediated inhibition of IL-1β release. Moreover, we showed that this mechanism depends on the release of a soluble low molecular weight factor.

Conclusions: We propose a novel anti-inflammatory mechanism induced by SLPI, which inhibits the ATP-dependent secretion of IL-1β. This novel signalling pathway might lead to the development of therapies needed for the treatment of inflammatory disorders.
Disease Area Cystic Fibrosis: Abstract No. 201 – 225
Abstract No. 201

The European Cystic Fibrosis Monozygous Twin Study

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The European Cystic Fibrosis Monozygous Twin Study Nadine Alfeis,1,2 Rebecca Hyde,1,2 Stephanie Tamm,1,2 Christian Dopfer,1,2 Jens Vogel-Claussen,2,3 Lutz Wiehlmann4 and Burkhard Tümmler1,21 Clinic for Pediatric Pneumology, Allergology and Neonatology, 3 Department of Diagnostic and Interventional Radiology, 4 Research Core Unit Genomics, Hannover Medical School, Hannover, Germany; and 2 Biomedical Research in Endstage and Obstructive Lung Disease (BREATH), German Center for Lung Research, Hannover, Germany

Twenty years ago the European Cystic Fibrosis Twin and Sibling Study (ECFTSS) had assessed the impact of environmental and genetic factors on the basic defect and the gastrointestinal and pulmonary phenotypes of cystic fibrosis (CF). By revisiting the monozygous CF twins of the ECFTSS we now would like to determine the influence of epigenetics and stochastic environmental factors on the basic defect and the CF disease presentation. CF twins are characterized in their basic defect (sweat test, NPD, ICM), anthropometry (height, BMI, impedance), lung disease (MBW, spirometry, MRI, microbial metagenome) and hematological features (cytometry, genome-wide methylome, IgG- and T-cell-receptor repertoire). At the DZL meeting we shall present the outline of the study, methodology and the first primary data.

01.12.2017 Nadine Alfeis
Abstract No. 202

**Identification of SLC26A9 chloride channel activators by high-throughput screening**

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**Introduction & Aims**

The SLC26A9 chloride channel represents a promising candidate to provide apical chloride transport in the absence of functional CFTR, thus circumvent the primary defect in cystic fibrosis. Recent evidence suggests that SLC26A9 Cl⁻ channel function may be activated therapeutically by compounds that increase translocation of the protein into the apical plasma membrane. To systematically identify therapeutic target genes and lead compounds promoting trafficking of SLC26A9, we aim to perform high-throughput siRNA and chemical library screens.

**Methods**

We generated CFBE41o- cells with stable expression of HA-tagged SLC26A9. To be able to measure chloride channel function on a high-throughput scale, we developed a live-cell microscopy-based assay using a membrane potential sensitive dye (FLIPR). Following siRNA-mediated knockdown, changes in membrane potential upon chloride channel inhibition by niflumic acid are measured by time-lapse imaging in 96-well format. Intensity time-traces for individual cells are quantified after segmentation and image quality control. Validation of the effect of siRNA silencing on chloride current is tested in Ussing chamber.

**Results**

Using the FLIPR assay we quantify the baseline and the response upon adding inhibitor. We were able to detect significant differences between fluorescence intensity changes of scrambled and SLC26A9 siRNA silenced cells. Transepithelial short-circuit current measurements showed that SLC26A9 knockdown significantly reduced the basal and the cAMP-stimulated chloride current, as well as it decreased the inhibition by niflumic acid.

**Conclusions/Perspectives**

We have developed a robust high-throughput assay to monitor SLC26A9 function. Currently we are testing a hypothesis driven siRNA library for SLC26A9 modulator genes. Hits from a primary screen can be readily validated by Ussing chamber. This platform will enable us in the future to identify therapeutic strategies to activate SLC26A9.
Abstract No. 203

One time quantitative PCR detection of Pseudomonas aeruginosa to discriminate intermittent from chronic infection in Cystic Fibrosis

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Chronic airway infection with Pseudomonas aeruginosa is major risk factor of progression of lung disease in patients with cystic fibrosis (CF). Chronic P. aeruginosa infection evolves from intermittent infection that is amenable to antibiotic eradication, whereas chronically adapted P. aeruginosa becomes resistant to antibiotic therapy. Discrimination of intermittent versus chronic infection is therefore of high therapeutic relevance, yet the available diagnostic methods are only partly satisfactory. The aim of the present study was, therefore, to evaluate the usage of quantitative PCR (qPCR) to measure pathogen abundance and to discriminate between intermittent and chronic Pseudomonas infection in patients with CF. Using an established qPCR protocol, we analyzed the abundance of P. aeruginosa in 141 throat swabs and 238 sputa from CF patients with intermittent or chronic infection with P. aeruginosa, as determined by standard culture based diagnostics. We observed a large increase of abundance of P. aeruginosa in throat swabs and sputum samples from patients with chronic compared to intermittent infections with P. aeruginosa. The data show that abundance of P. aeruginosa as measured by qPCR is a valuable tool to discriminate intermittent from chronic infection. Of note, P. aeruginosa burden seems more sensitive than mucoidity phenotype to discriminate chronic from intermittent strains. Furthermore we observed that molecular detection in throat swabs was linked to a viable culture in the sputum when sputum was available. This result is of special interest in young patients with cystic fibrosis that often cannot expectorate sputum. We also observed that qPCR in comparison to culture detected the infection earlier. The results suggest that qPCR detection and quantification of P. aeruginosa is a precious tool to be added to the diagnostic toolbox in cystic fibrosis.
Abstract No. 204

Longitudinal analysis of the airways’ microbiota in patient with Cystic Fibrosis

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In patients with cystic fibrosis, several next generation sequencing based studies were established in the last decade to decipher cross-sectionally the structure and composition of the airways’ microbiota. The aim of our study was to analyze the evolution of the microbiota among the airways (nose, throat and lung). Using 16S amplicon sequencing, we analyzed 87 nose swabs from 10 patients, 462 throat swabs from 43 patients, and 763 sputum samples from 60 patients for an average time period of 3 years. We observed that each patient possessed a personalized microbiome as the Morisita-Horn distance between patients’ related microbiomes was lower than between non-related microbiomes for the three airways’ compartments. Our results also showed that the establishment of a chronic infection by P. aeruginosa was increasing the instability of the throat microbiome while it was decreasing the instability in the sputum indicating that the establishment of the infection in the lower airway correlates with a dysbiosis in the upper area. P. aeruginosa infection in the lower airway was also correlated to an accelerated decline of the alpha-diversity per year as well as Staphylococcus infection. In contrast, anaerobes (Prevotella and Veillonella) were correlated with a stabilization of the decline in the alpha-diversity per year. Finally, we observed that dominance was correlated to an increase of the stability of the microbiome over the time indicating a failure in the elimination process by the lung. In conclusion, our results show that the evolution of the microbiome in the lower airway is mostly dependent of the acquisition of a chronic infection by a CF pathogen which is correlated with a break in the dynamic colonization/elimination of the lower airways. On the other side, the maintenance of anaerobes in the lower airways was correlated to a more dynamic microbiome with a stable/increased alpha-diversity over the years.
Abstract No. 205

**Characterization of Airway and in vitro Differentiated Macrophages in a Mouse Model of Cystic Fibrosis**

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Airway macrophages play a major role in the first line of defense of the innate immune system of the lungs. In cystic fibrosis (CF) this function is even more important since there is a chronic and excessive airway inflammation triggered by neutrophils. During the last years, an increasing number of publications gave hint that mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene not only cause malfunctions of the Cl- and bicarbonate CFTR channel in epithelial cells but in macrophages as well. Own data in a Cftr mouse model revealed that transplantation of hematopoietic stem cells from wild type mice had a beneficial effect in these chimeric CF mice under infectious conditions compared to CF mice transplanted with isogenic stem cells. FACS analysis of macrophages from lungs of chimeric mice showed an almost complete replacement of the original CF macrophages by the transplanted wild type cells, which engrafted successfully in the lungs. These data were enhanced by immunofluorescence of the lungs of chimeric mice, where transplanted cells could be detected and stained with typical macrophage markers. In parallel, we set up an assay to evaluate differences in the pH in in vitro differentiated macrophages of CF and wild type mice measuring fluorescence with the pH-sensitive dye LysoSensor Green DND-189 via confocal microscopy. Our results confirmed the hypothesis of an impaired acidification in lysosomes of CF macrophages compared to healthy ones. As a next step, we are going to investigate macrophages of CF mice transplanted with wild type hematopoietic stem cells to find out, if this approach restores malacidification in the airway macrophages and might be of therapeutic interest for CF patients to fight for their chronic lung infections.
Abstract No. 206

Relative roles of mucus dehydration and mucin hypersecretion in the in vivo pathogenesis of airway mucus obstruction

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\textbf{Background:} Airway mucus obstruction is a hallmark of chronic lung diseases such as cystic fibrosis (CF), chronic obstructive pulmonary disease (COPD), and asthma. Mucus dehydration and mucin hypersecretion have been implicated in the pathogenesis of airway mucus plugging, however, understanding of the relative contributions and combined effects of these abnormalities in muco-obstructed lung diseases remain poorly understood.

\textbf{Methods:} To determine the relative roles of mucus dehydration and mucin hypersecretion, we compared mice with airway surface dehydration due to airway-specific overexpression of the β-subunit of the epithelial sodium channel that lack the mucin-inducing cytokine IL-13 (\textit{Scnn1b-Tg/Il13\textsuperscript{-/-}}) to mice with airway-specific overexpression of IL-13 (\textit{Il13-Tg/Il13\textsuperscript{-/-}}). Further, we studied mice exhibiting both defects (\textit{Scnn1b-Tg/Il13-Tg/Il13\textsuperscript{-/-}}).

\textbf{Results:} We found persistent severe airway mucus plugging in the absence of goblet cell metaplasia (GCM) and increased mucin expression in juvenile \textit{Scnn1b-Tg/Il13\textsuperscript{-/-}} mice. Despite exaggerated GCM and \textit{Muc5ac} and \textit{Muc5b} expression in \textit{Il13-Tg/Il13\textsuperscript{-/-}} mice, mucus plugging was significantly decreased compared to \textit{Scnn1b-Tg/Il13\textsuperscript{-/-}} mice. This observation was associated with hyperabsorptive epithelial ion transport and impaired mucociliary transport in \textit{Scnn1b-Tg} mice, and a hypersecretory phenotype and normal mucociliary transport in \textit{Il13-Tg/Il13\textsuperscript{-/-}} mice. In neonatal \textit{Scnn1b-Tg/Il13-Tg/Il13\textsuperscript{-/-}} mice, elevated mucin expression did not differ from \textit{Il13-Tg/Il13\textsuperscript{-/-}} mice. However, the combination of mucus dehydration and mucin hypersecretion caused severe mucus plugging and neonatal mortality in these mice.

\textbf{Conclusions:} Our data provide novel insights into the relative roles of mucus dehydration vs. mucin hypersecretion and demonstrate that in dehydration-mediated mucostatic airways, mucin hypersecretion aggravate mucus plugging leading to invariable neonatal death indicating that both defects have detrimental effects in the \textit{in vivo} pathogenesis of muco-obstructed airways.

Supported by the German Federal Ministry of Education and Research (82DZL004A1 and 82DZL004A1).
Abstract No. 207

The adaptation-process of Pseudomonas aeruginosa in CF-lungs: Correlation between phenotype and genotype

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Chronic airway infections with P. aeruginosa typically determine the clinical course of patients suffering from CF. During chronic infection the bacteria undergo microevolution which supposedly reflects the adaptation to the CF lung habitat and to the antimicrobial treatments administered over the years. Here, the adaptation process of P. aeruginosa was observed in sequential isolates of the initially colonizing clone from twelve CF-patients, six with a mild and six with a severe clinical course. Therefore, the isolates were analyzed pheno- and genotypically. More than 250 sequential isolates were characterized in mutation rates and phenotypic traits such as morphology, motility and virulence effector secretion. Of this pool of strains isolates were chosen in one year intervals and sequenced by next-generation sequencing. Genetic peculiarities such as nucleotide variations, small indels and larger deletions compared to the initial isolate were extracted and used for clade reconstruction. Hotspots of mutations were determined. Contrary to what is written in the literature box plots of phenotypes displayed no clear loss of motility or siderophore secretion over the course of infection. For all analyzed phenotypic traits, except for swarming, a broad range of characteristics could be verified. Phenotypes were not linked with clades. Overall, genes associated with antibiotic resistance and adaptation were overrepresented among hot spots of mutations. To see if those mutations are functional, various bioassays are planned for the future.
Abstract No. 208

Intraclonal competitive fitness of sequential P. aeruginosa strains from CF-patients differs by the clinical severity of infection

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The common Gram-negative bacterium P. aeruginosa is an important causative agent in nosocomial infections and the pathogen which contributes most to the shortened life expectancy of CF-patients. In this work we wanted to analyze if there is a fitness advantage or disadvantage due to pathoadaptive mutations in the CF-lung. Therefore, serial clonal P. aeruginosa isolates taken from 12 CF-patients (six patients with a mild and six with a severe course of infection) were competitively grown together in different media to mimic environmental and CF-lung conditions. Samples were taken at 0 h, 48 h (with continuous culturing every 12 h) and 120 h (without continuous culturing), DNA was prepared, multiplex PCR was performed and resulting amplicons were sequenced. The percentage of each isolate within a sample was calculated based on isolate specific SNPs. In nearly all 12 courses one or two strains could outcompete all others. For the severe courses we observed in all cases that the first isolates outcompeted the later isolates, whereas in the mild courses no tendency could be detected. For some of these winner strains we identified SNPs, stop-mutations, deletions or extra genes which could be directly linked to a fitness advantage. The late isolates of the severe courses acquired more drastic genomic changes which caused a growth disadvantage compared to the non-adapted first isolates.
Abstract No. 209

**Hot spots of side-by-side sequence and RGP variants in Pseudomonas aeruginosa strains.**

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Early antimicrobial treatment has been established as an efficacious measure to eradicate *P. aeruginosa* from CF airways when it is detected for the first time by culture-dependent diagnostics.

Thanks to the recent developments of next generation sequencing technologies, we got an in-depth insight into the intraclonal diversity of *P. aeruginosa* in CF lungs during the onset of colonization that is not observable by any established genotyping method such as genome fingerprinting, oligonucleotide microarray or Sanger-based multilocus sequence typing. With the accurate SOLiD technology, we were able to detect subclonal variants within one strain whereby the two nucleotides of a SNP were commonly present in different proportions in pre- and post-treatment samples indicating a variable composition of SNPs variants in *P. aeruginosa* colonies. Most subclonal SNPs do not change the coding sequence and may be classified as neutral substitutions that do not confer any advantage or disadvantage to the bacteria. The exceptions from the rule were missense and stop mutations in lasR. The co-existence of LasR isoforms may reflect diversifying selection to improve the fitness of *P. aeruginosa*.

A variation was also recognized during the annotation of RGPs of the clone C genome. Resequencing of the same isolate using SMRT sequencing showed the same within strain variation for RGPs showing that also larger blocks of DNA are varying within a strain.
Abstract No. 210

**Spectrum of mutations in pathoadaptive Loci of *P. aeruginosa*.**

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Pathoadaptive loci are defined as those genes that acquire a high number of mutations during persistence in a chronic patient habitat like the CF lung. Twenty genes including *lasR, nuoL, algG, pelA* and *pelF* were identified as pathoadaptive loci in a set of 12 CF patient courses (6 mild and 6 severe). The aim of this project is to screen how mutations in the investigated loci are distributed in environmental, acute infection and cross-sectional chronic infection isolates. Using amplicon sequencing on an Illumina NextSeq we were able to sequence the pathoadaptive loci of more than 500 isolates (66 acute infections, 98 environmental, 375 chronic infection isolates) in a fast and cost effective manner. After filtering for decent coverage and read distribution we were able to detect a various number of mutations in the strain panel. The range of mutations in a loci varied between a dozen and more than 100 candidate positions whereby some rare positions seem to be specific for one habitat. Both synonymous and non-synonymous mutations seem to be present in the same amount of affected positions within the genes with the limitation that neutral mutations tend to be present in more isolates. An example for a rare mutation is an early Stop mutation in *algG* only found in two chronic infection isolates.
Abstract No. 211

Monitoring of protease activity in airway inflammatory cells of Cystic Fibrosis patients using FRET sensors

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Neutrophil Elastase (NE) is a hallmark for the development and progression of cystic fibrosis (CF) lung disease. Free NE activity in sputum and bronchoalveolar lavage (BAL) fluid is identified as major risk factor causing decline in lung function and early stage bronchiectasis. Nowadays research focuses mainly to measure free NE activity in supernatants and BAL fluids. Within this study measurement of several clinical parameters like forced expiratory volume in 1 second (FEV1) should be longitudinal compared to free and membrane bound protease activity as well as cytokine levels. Recently, lipidated FRET (Förster resonance energy transfer) reporters were developed which allow a detection of NE activity on cell surfaces. To get reliable results of the membrane associated NE activity over a longer period of time the existing protocol was improved to be able to measure several patients (2-5) a week. So far the spontaneous sputum of 70 CF patients (10 treated with CFTR modulator, 16 exacerbated) could be collected. Cells were isolated and separated from the supernatants; the membrane-associated NE activity was quantified with the FRET reporter NEmo-2 within 2h after cough up. The activity was calculated as donor/acceptor ratio and normalized to the samples treated with Sivelestat (an NE inhibitor). A first subset of patients, not discriminating between exacerbated and stable patients, was correlated with clinical data. Membrane-associated NE activity of CF patients moderately correlates with airflow limitation (FEV1 predicted, rho=0.48, p<0.01, n=37) and highly with air trapping (FRCpleth predicted, rho=0.61, p<0.01, n=23). The measurements of free NE activity, cytokines (IL-1β, IL-6, IL-8 and TNF-α) and anti-proteases (SLPI, NEAAT and TIMP-1) are ongoing, free proteinase 3 and cathepsin G will be included in the near future. 25 patients were already measured more than once (2-4 times), we are aiming to get longitudinal measurements of at least 40 different patients.
Abstract No. 212

Clinical implications of the cystic fibrosis airway microbial metagenome

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Background Although some common nosocomial pathogens are known to be the prevalent microbes in CF lungs we have learnt during the last years that CF lungs are inhabited by a complex polymicrobial community. However, the pathogenic role of these many facultative or strict anaerobes still needs to be clarified.

Aims Based on a large collection of upper and lower airway CF microbial metagenomes the association of individual genera and/or species with disease status was investigated.

Methods Three types of respiratory tract secretions from 71 patients of the Hannover CF Clinic were subjected to high-throughput shotgun whole genome sequencing. Patients' BMI and FEV1 values at the day of sample collection and the nine months prior to and after this date were converted to centiles based on the age- and sex-dependent reference centile distribution of the ECFS registry population. Presence and abundance of genera or species were mapped onto the patient's position defined by disease centile and age.

Results Of the microbial metagenome sequencing datasets, one dataset was randomly selected leading to a collection of 48 induced sputum samples, 29 oropharyngeal swabs and 48 nasal-lavage specimens. Patients harbored very diverse microbiomes in the three airway compartments. Total microbial load was not associated with disease severity. Besides the common CF pathogens the normal airway inhabitants like Streptococci, Rothia, Veillonella and Prevotella were prevalent in large amounts.

Prevotella melaninogenica showed significant association with health in PI Patients ($P = 0.03$).

Conclusion This cross-sectional study provided insight into the association between microbiome composition and age and disease status of CF patients. Our data suggest that $P.$ melaninogenica which is typically not recovered by current protocols of culture-dependent diagnostics could serve as a biomarker of CF patient’s health in the future.

Acknowledgments. MG is a member of the Klin.StrucMed programme financed by the Else-Kröner Stiftung.
Abstract No. 213

Investigating Neutrophil Serine Proteases activity at subcellular resolution

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Cathepsin G (CG), Neutrophil Elastase (NE) and Proteinase 3 (P3) are the three neutrophil serine proteases (NSP), the eclectic players of neutrophil-mediated inflammation and pathogen fight. They are stored in primary granules and, responding to bacterial infections, they are compartmentalized inside phagolysosomes where they help digesting microorganisms. After neutrophils degranulation, they can also translocate to the cell surface, here they carry out their antimicrobial and inflammatory regulation activities. Finally, bacteria and platelets activate the secretion of DNA web-like structures able to prevent the spreading of pathogens (NETosis), and the resulting neutrophil extracellular trap (NET) is studded with NSP.

The NSP are active at these three different cellular locations, and an uncontrolled increase in their activity has been associated with the \textit{in vivo} pathogenesis of lung diseases as chronic obstructive pulmonary disease (the fifth leading cause of death in the world) and cystic fibrosis (the most common lethal genetic disease affecting Caucasian population). Both conditions result in a massive neutrophilic airway inflammation promoting extensive and non-reversible tissue damaging due to uncontrolled NSP release.

Visualization and quantification of the NSP activities and interplay at subcellular resolution would shed light on their biology, it may unveil new biomarkers for disease prediction outcome and could be used for current therapies evaluation. Here, we propose the development of a new series of ratiometric FRET reporters: a lipidated CG reporter to visualize its activity on the plasma membrane, a soluble CG probe for recording the enzyme action in patients sputum supernatant an engulfable FRET beads that allow to monitor CG inside phagosomes and a NET associated probe.
Abstract No. 214

Absence of T cells reduces structural lung damage in an IL-17A-dependent manner in mice with cystic fibrosis-like lung disease

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Little is known about the contribution of the adaptive immunity in the in vivo pathogenesis of cystic fibrosis (CF) lung disease and its impact on chronic airway inflammation, mucus plugging and structural lung damage. Mice with airway-specific overexpression of the β-subunit of the epithelial sodium channel (Scnn1b-Tg) mimic pathogenic features of CF lung disease.

To investigate the role of adaptive immune cells in the in vivo pathogenesis of CF-like lung disease, we crossed Scnn1b-Tg mice with RAG1-deficient (Rag1-/-) mice lacking mature T and B cells and compared inflammatory cell counts, mucus obstruction, structural lung damage, gene expression and cellular sources of IL-17A in juvenile Scnn1b-Tg mice with Scnn1b-Tg/Rag1-/- mice, and their wild-type (WT) and Rag1-/- littermates.

Inflammatory cell counts were elevated to similar levels in Scnn1b-Tg and Scnn1b-Tg/Rag1-/- mice. Mucus obstruction and elevated mucin expression were independent of the presence of T and B cells in Scnn1b-Tg mice. Structural lung damage was significantly reduced in Scnn1b-Tg mice lacking T and B cells. Gene expression of IL-17A, a cytokine associated with structural lung damage, was significantly elevated in lungs from Scnn1b-Tg mice compared to WT mice and mice lacking T and B cells. We identified γδ T, CD4+ T cells and ILC3s as major sources of increased IL-17A levels in lungs of Scnn1b-Tg mice. Finally, we also observed reduced structural lung damage in Scnn1b-Tg mice that lack IL-17A expression.

Our results suggest that adaptive immune cells do not contribute primarily to the pathogenesis of chronic airway inflammation and mucus obstruction in CF-like lung disease. However, absence of T cells in Scnn1b-Tg mice reduces structural lung damage which could be related to decreased levels of IL-17A and that might also be a potential pathomechanism in human CF lung disease.

Supported by BMBF (82DZL00401 and 82DZL004A1).
Abstract No. 215

ICM/npD Index cases: Diagnostic features of subjects with CFTR-related disorder

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By 2011 the CF Electrophysiology Laboratory at MHH established computer-assisted recording of nasal transepithelial potential (NPD) and intestinal current measurements (ICM) according to the SOPs of CFFT and ECFS. During that period from January 2012 to December 2017 209 subjects with non-informative sweat test and/or inconclusive CFTR mutation analysis were sent from external physicians to make a diagnosis by NPD and/or ICM. In total 21 subjects were diagnosed to suffer from PS CF, 52 subjects were diagnosed to be affected by a CFTR-relater disorder. We would like to discuss the outcome of CFTR diagnostics of typical borderline cases in the light of the current algorithm:

Two 5-year & 8-year old siblings were compound heterozygous for p.Phe508del and the complex allele of the three mutations p.Arg74Trp-p.Val201Met-p.Asp1270Asn, each of which classified as a sequence variant of unknown clinical significance. The CFTR triple mutant was found to be attenuated in synthesis and function. Five subjects presented CF-like symptoms of the respiratory tract, non-informative CFTR mutation genotypes, normal sweat Chloride and aberrant tracings in the NPD. A 60-year old subject with bronchiectasis was diagnosed to suffer from CF by pathological NPD tracings typical for CF on the occasion of the first presentation at MHH’s lung transplantation clinic(BMI 18, FEV1 37%; P. aeruginosa in sputum). No mutation was identified by sequencing of the CFTR coding region. A mother(38 years) and her two daughters (15 years,17 years) who carry no mutation in the CFTR coding region, presented matching clinical symptoms(chronic sinusitis, episodes of pancreatitis) and NPD tracings(reduced CFTR conductance) suggesting an autosomal dominant trait of reduced CFTR activity. These index cases demonstrate the diverse etiology of CFTR dysfunction in individuals with mild CF or CFTR-related disorder.

Rebecca Hyde, 27.11.2017
Abstract No. 216

The microevolution process of Pseudomonas aeruginosa in CF-lungs: genomic analysis

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Chronic airway infections with \emph{P. aeruginosa} typically determine the clinical course of cystic fibrosis (CF) patients. During chronic infection the bacteria undergo microevolution while adapting to the CF lung habitat and antimicrobial treatment over the years.

We analysed the \emph{P. aeruginosa} microevolution in sequential isolates from six CF-patients with a mild and six with a severe clinical course. Serial isolates were genome sequenced to determine the genomic variations that occurred during the habitat colonisation; the isolates were also tested for several phenotypes and competitive fitness.

The sequential isolates displayed modest mutation rates unless hypermutator phenotypes manifested early in the infection course. Most mutations were nucleotide exchanges or small indels but also DNA loss or uptake could be detected. Phylogenetic trees based on the detected variations illustrated the underlying microevolution and displayed a spectrum of modes, ranging from maintenance of a single adapted strain to long-term persistence of co-existing clades with mixed types in between. Trees for severe courses isolates revealed an increased frequency of extinct clades while for mild courses higher proportions of persisting clades were seen, indicating a trend to more diversifying evolution in bacteria from mild CF courses. Differences were also detected for mutation types, as stop and frameshift mutations were more prominent in severe courses isolates. These findings provided examples for the potential influence of the patient status on bacterial microevolution in CF airways.
Abstract No. 217

**Slc26a9 deficiency causes high neonatal mortality due to airway mucus plugging**

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The alternative Cl- channel SLC26A9 has been associated with a higher risk of developing Cystic Fibrosis (CF), bronchiectasis and asthma, supporting SLC26A9 as a modifier of a spectrum of muco-obstructive lung diseases. The aim of this project was to determine the in vivo role of SLC26A9 during early postnatal adaptation. We, therefore, compared the pulmonary phenotype of wild-type (WT) and Slc26a9 deficient (Slc26a9-/−) mice including survival, histology, mucin transcript levels, µCT imaging analysis, wet/dry lung weight and oxygen saturation measurements. At birth, all genotypes were represented according to Mendelian genetics. Nevertheless, within 30 minutes after birth Slc26a9-/− mice presented respiratory distress and cyanosis, resulting in 48% mortality compared to WT mice (p<0.05). To investigate whether this was due to a lung liquid clearance dysfunction, wet/dry measurements were performed. Here, no differences were found between the two genotypes (p=0.56). To determine the cause of death, histological analyses were performed and revealed mucus plugging in the trachea, proximal and distal airways in deceased Slc26a9-/− mice compared to WT mice (p< 0.01 for all levels analyzed). The expression of Muc5ac and Muc5b were also elevated in deceased Slc26a9-/− mice compared to WT mice (p< 0.05). By measuring the effect of mucus plugging on ventilation Slc26a9-/− mice showed significantly reduced oxygen saturation of 32% compared to 80% in WT mice (p<0.01). Preliminary results from µCT studies support early onset airway mucus obstruction associated with atelectasis in Slc26a9-/− mice. Taken together, our data support that SLC26A9 plays an important role in airway mucus clearance in the neonatal lung and that this process is essential for normal postnatal adaptation. Supported by the BMBF (82DZL00401 and 82DZL004A1)
Abstract No. 218

**Airways microbial metagenome of individuals with immune deficiency, asthma, cystic fibrosis or COPD**

Katarzyna Pienkowska¹, Marie Dorda¹, Lutz Wiehlmann¹, Frederik Behr¹, Silke Hedtfeld¹, Rebecca Hyde³, Ulrich Baumann¹, Gesine Hansen¹, Olaf Holz¹, Jens Hohlfeld¹, Joachim Mainz¹, and Burkhard Tümmler¹

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Introduction.
Airway infections cause exacerbations of the underlying disease in patients with immune deficiency, asthma or COPD and determine course and prognosis in most individuals with cystic fibrosis.

Objectives. We wanted to identify the composition of the upper and lower airway metagenome in these patient groups in a clinically stable condition.

Methods.
Nasal lavage, throat swabs and sputa were collected from 142 CF, 35 asthma, 11 COPD and 10 ID patients. Genomic DNA was sequenced by either SOLiD or Illumina technology. After quality filtering of the raw reads human and low complexity reads were removed. The remaining reads were mapped onto a microbial pangenome of completely genomes of 1.892 bacteria, 4.193 fungi and 1.153 moulds and 1.153 DNA viruses. Results were corrected for the GC-bias and normalized to genome size of the organism and DNA content of one human cell. The program MetaPhlAn2 was used for taxonomic classification and construction of heatmaps. Phylogenies were constructed with the tool GraPhlAn.

Results.
COPD patients exhibited an airway metagenome dominated by Streptococci, Rothia and Prevotella similar to healthy smokers and non-smokers. Pre-school wheezers were mainly carrying a normal, but low-diversity microbial flora with Rothia mucilaginosa as prime and Actinomycetes as diagnostic organisms. Besides numerous aerobes and anaerobes, patients with ID were harbouring Moraxella and Haemophilus as major pathogens. Individuals with CF exhibited a disease-specific flora primarily made up of Firmicutes (Staphylococcus spp.), alpha- and gamma- Proteobacteria (P. aeruginosa, H. influenzae, S. maltophilia). Principal component analysis uncovered clusters of species that were associated with the severity and chronicity of CF lung disease.

Conclusion.
Disease-associated bacterial metagenomes were seen in patients with CF or ID, but not in patients with asthma or COPD. The prevalent phyla in human airways are Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria which make up more than 90% of the metagenome.
**Inhibition of STAT3 improves the processing and function of the Cystic Fibrosis protein CFTR**

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We have previously shown that decreased amounts of STAT3 mRNA are associated with higher levels of CFTR-mediated residual chloride secretion in the intestinal tissue of F508del-CFTR homozygous patients [1]. We have compared the CFTR gene sequence at variant positions and could describe differential STAT3 binding to the two contrasting alleles at the single nucleotide polymorphism J3.11. Six commercially available STAT and specific STAT3 inhibitors [2] were used to interfere with STAT3 function in model epithelial cell lines 16HBE14o- and T84. Lack of solubility in non-DMSO solvents was observed for three inhibitors and cell toxicity could be observed for two chemicals. Strikingly, using independent results from two investigators and two batches of substances, a consistent upregulation of mature, fully glycosylated CFTR was observed in both cell lines upon treatment with three inhibitors reported to target specifically STAT3. While STAT3 is a central molecule that transduces many cellular signals, suggesting that a therapeutic intervention with STAT3 inhibitors can only be attempted if side effects are amenable to control, this proof-of-principle experiment demonstrates that CFTR function is influenced by the STAT3-mediated cytokine signaling network [3] and might help to explain why genes that determine immunology and inflammation modify the basic defect of impaired ion conductance in cystic fibrosis epithelia [4].


This project is funded by the Mukoviszidose Institut gGmbH (#MI-1601) and supported by the DZL. E.R. has received a StrucMed stipend from the DZL, partner site BREATHE.
Abstract No. 220

Allergic sensitization against classic allergens in patients with cystic fibrosis as measured by component-resolved analy

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Background: Sensitization against the mould Aspergillus fumigatus, in its most pronounced form presenting as allergic bronchopulmonary aspergillosis (ABPA), is well known in patients with cystic fibrosis (CF). Allergic sensitization against classic allergens is less well researched. Chip-based, component-resolved diagnostics to detect sensitization against classic allergens might discover hitherto unknown sensitization patterns in patients with CF.

Methods: As a pilot project to delineate sensitization patterns in 28 CF patients (56.3% male, mean age 16.06 ± 8.97 years), we measured sensitization against 81 classic allergens by component-resolved ISAC-chip technology divided into two groups according to total IgE (tIgE) level (elevated vs. normal age-dependent tIgE).

Results: Sixteen out of 28 CF patients had elevated tIgE levels among which 9 patients suffered from ABPA. Patients with elevated tIgE displayed a mean of 7.5 specific sensitizations (cut-off 0.7 immuno-solid-phase allergen chip standardized units, ISU, maximum number of sensitizations 25) while patients with normal tIgE showed a mean of 1.58 specific sensitizations (maximum 12). Specific sensitization against Aspergillus fumigatus occurred in 7/16 of patients with elevated tIgE vs. 0/12 patients with normal tIgE. Patients with normal tIgE also showed less sensitization against bermuda (5/16 vs. 1/12) and timothy grass (6/16 vs. 3/12) and tree pollen (birch, grey alder) and peanut (all 4/16 vs. 1/12). Specific IgE against house dust mite and Alternaria alternata was not different among groups with elevated vs. normal tIgE.

Conclusions: Our results show markedly different sensitization patterns of CF patients vs. healthy controls and suggest differences in sub-groups of CF patients. They can provide an initial basis for a risk profile of sensitization for development of ABPA. In this context, prospective studies are needed to decipher the association of sensitization patterns and ABPA development but also to query the effect of allergic sensitization on disease course in CF beyond ABPA.
Abstract No. 221

**Non-CF related CRS and CF-related CRS**

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Patients with cystic fibrosis (CF) frequently suffer from chronic rhinosinusitis (CRS), a chronic disorder of the upper airways characterized by nasal congestion and discharge. CRS is characterized by signs of sinonasal inflammation, epithelial remodeling and impaired mucociliary clearance. The knowledge about the pathophysiology is limited. Here, we aimed to study and compare the epithelial ion transport in cultured nasal epithelia of CRS patients with CF and without CF. Human nasal epithelial primary cells (hNEpC) were freshly isolated from nasal tissue from the paranasal sinuses of CRS patients w/o CF undergoing polyps resection. HNEpC were cultured for 14 days and transepithelial short-circuit current (Isc) was measured in Ussing chambers under a Cl- gradient. Functional evaluation of hNEpC cultures from CRS patients (non-CF) revealed a significant reduction in the basal Isc (p<0.01), the amiloride-sensitive Isc (p<0.01), the amiloride-insensitive current reflecting constitutive Cl-secretion (p<0.01) as well as the UTP-induced Isc accounting for Ca2+-mediated Cl-secretion (p<0.05) compared to healthy individuals. In hNEpC cultures from CRS patients with CF (n=8), bioelectric studies exhibited a different picture. The UTP-induced Isc was significantly increased compared to non CF-related CRS cultures (p<0.05). The cAMP-induced Cl- secretion reflecting CFTR activity was significantly decreased in CF-related CRS cultures (p<0.01). In summary, an abnormal epithelial ion transport driven by a loss of Cl-secretion namely the UTP-induced responses in non-CF related CRS was observed. In CF-related CRS, changes in ion transport were due to the genetic loss of CFTR function, whereas no loss of UTP-induced currents was observed. Further studies analyzing the Ca2+-activated Cl- secretory pathways are required to draw a better picture of pathophysiologic mechanisms in non-CF related and CF-related CRS. Supported by BMBF (82DZL00401).
Abstract No. 222

The Interleukin 1 Receptor is a Genetic Modifier of Cystic Fibrosis

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Recent studies identified IL-1 receptor (IL-1R) signalling as an important pathway triggering neutrophilic airway inflammation that constitutes a key risk factor in the onset and progression of lung disease in patients with CF. The aim of this study was to assess the role of the IL1R gene as a modifier of disease severity in patients with CF. We interrogated the IL1R locus with the informative microsatellite marker IL1RSat in several independent patient cohorts and a non-CF population control. We compared the distribution of IL1RSat allele frequencies between the inherited genetic information transmitted to the CF patients to the information that was retained in the parental generation from families of The European CF Twin and Sibling study, detecting a transmission disequilibrium with allele 15 at IL1RSat overrepresented in the recruited CF sibs (pTDT = 0.0083) which annotates ILR1Sat allele 15 as a benign variant associated with survival of CF patient until enrolment into the study. In two independent cohorts of CF patients that were born before 1970 and survived until at least 1990, IL1RSat alleles 14 and 15 were the most frequently observed alleles, albeit this trend towards a similar pattern of enrichment of allele 15 at IL1RSat did not reach statistical significance (Pbest = 0.08) with the number of patients that were available for this study. This genetic association study suggests that IL-1R is a significant modifier of disease severity in patients with CF. Our finding that the IL1R locus conveys a survival advantage for patients with CF strongly supports an involvement of IL-1R in CF pathogenesis. Together with recent physiological studies, these findings also indicate that inhibition of IL-1R signalling may be a promising anti-inflammatory strategy in CF and potentially other lung diseases associated with chronic airway mucus obstruction and neutrophilic inflammation.
Abstract No. 223

The transcription factor EHF and the MGAT protein glycosylation enzymes determine the processing of the Cystic Fibrosis protein CFTR in epithelial cells

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The gene encoding the epithelial-specific transcription factor EHF was identified as a CF modifying gene in a genome-wide association study [1]. The genetic background of EHF was associated with CFTR-mediated residual chloride secretion among F508del-CFTR homozygous cystic fibrosis patients [2]. Genes regulated by EHF in A549 cells control wound healing [3] and the expression profile of rectal biopsies from F508del-CFTR homozygous patients suggest that MGAT enzymes that mediate complex protein glycosylation and trafficking [4,5], are under the control of EHF [2]. The aim of this study was to validate the role of EHF and MGATs for CFTR processing by functional analyses within model epithelial cell lines. siRNA was used to downregulate EHF or its suspected target genes MGAT1, 2, 3 and 4a in 16HBE14o- and T84 cells. mRNA analysis of 16HBE14o- cells treated with siRNA directed against EHF has confirmed that the expression of EHF and of MGAT transcripts are interrelated. Furthermore, the expression levels of fully glycosylated mature CFTR protein is decreased in both cell lines upon treatment with siRNA directed against EHF or MGAT enzymes. Taken together, these data show that the trafficking and processing of CFTR as a complex-glycosylated membrane protein at the apical membrane of epithelial cells is determined by key enzymes of the Golgi network that control the formation of complex glycosylated proteins and that EHF directly influences the maturation of CFTR in epithelial cells.


This project is funded by the Mukoviszidose Institut gGmbH ( #MI-1503) and supported by the DZL.
The TNFα receptor TNFR1 is known for its immunomodulatory and apoptotic effects; however it was recently noticed that this gene modulates disease severity and the CFTR-mediated basic defect of residual chloride conductance in Cystic Fibrosis. [1] Two contrasting genes of TNFR1 in F508del homozygous patient pairs have been described. We now want to validate these results by a replication study in a present-day cohort of unrelated CF patients. Therefore, F508del-CFTR homozygous patients who had a valid nasal potential difference measurement to characterize their basic defect manifestation in the past, as well as their parents, are recruited at the DZL centres BREATH, TLRC and UGMLC. TNFR1 genotyping with 5 genetic markers is performed either with DNA samples already stored in the biobank or the DNA is collected by the “Oragene saliva DNA self-collecting kit”. Several studies tested the kit before [2] and the sample tubes can easily be sent and received by mail. By 11/2017, 24 out of 31 sample tubes have already been returned.

All of the genetic makers that defined the contrasting TNFR1 alleles are located in the intron 1 of TNFR1. In the ENCODE project database mRNAs highly similar to the intron 1 sequence can be found, suggesting that there may be alternative transcript isoforms of the TNFR1 which still contain regions of intron 1. To verify this hypothesis, we currently perform combinatorial PCR to check for alternative transcripts in cDNA of cell lines 16HBE40o- and T84. Alternative transcripts might explain why TNFR1 is a genetic modifier of Cystic Fibrosis and how variant TNFR1 alleles influence the cellular phenotype.


This project is supported by the DZL. AU receives a StrucMed stipend from the DZL since 08/2017.
MMP-9 deficiency does not alter disease progression in mice with cystic fibrosis-like lung disease

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Cystic fibrosis (CF) lung disease is characterized by chronic neutrophilic airway inflammation which proceeds to proteolytic lung tissue destruction. Increased neutrophil elastase (NE) levels were identified as risk factor for the onset of structural lung damage and NE activated pro-matrix metalloproteinase (MMP)-9 led to the progression of bronchiectasis in young CF patients. It has been shown that mice with airway-specific overexpression of the beta subunit of the epithelial sodium channel (βENaC-Tg) share key features of CF lung disease such as chronic inflammation, lung tissue destruction and impaired lung function. We recently demonstrated that the deletion of NE in βENaC-Tg mice led to reduced neutrophilic inflammation and emphysema severity. To determine the role of the NE effector, MMP-9, in the progression of CF lung disease, we used genetic deletion of MMP-9 in βENaC-Tg mice and studied the effects on airway inflammation and emphysema. Phenotype alterations were analyzed in 6-8 week old mice by inflammatory cell counts in bronchoalveolar lavage (BAL) fluid and mean linear intercepts (MLI) assessment. Further, lung function was assessed in βENaC-Tg/NE-/- and βENaC-Tg/Mmp9-/- and compared to βENaC-Tg mice using a flexiVent apparatus. Neutrophil counts were equal in βENaC-Tg and βENaC-Tg/Mmp9-/- mice and both significantly elevated compared to WT mice. Mean linear intercepts indicated no significant difference between βENaC-Tg/Mmp9-/- and βENaC-Tg mice. Lung function measurement showed an improvement in the inspiratory capacity and static compliance in βENaC-Tg/NE-/- compared to βENaC-Tg mice. MMP-9 deletion had no significant influence on lung function in βENaC-Tg mice. The current study suggests that MMP-9 is not a crucial factor in the in vivo pathogenesis of CF lung disease. Inter-species differences in particular a dominant neutrophilic inflammation in humans and higher levels of antiproteases in mouse lungs might account for the contrasting observations. Further studies are needed to address the differences between patients and mice.
Disease Area Pneumonia and Acute Lung Injury: Abstract No. 301 – 331
Abstract No. 301

TGF-β impairs protein clearance by promoting phosphorylation, endocytosis, ubiquitination and degradation of megalin in alveolar epithelial cells

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Disruption of the alveolar-capillary barrier leads to elevated alveolar protein concentrations and is a distinctive feature of acute respiratory distress syndrome (ARDS). We have previously shown that megalin, a 600 kDa endocytic receptor plays a central role in alveolar clearance of excess protein and that TGF-β, a key player in the pathogenesis of ARDS induces GSK3-β-dependent megalin phosphorylation thus reducing alveolar protein clearance. In the current study, we investigated the molecular mechanism by which TGF-β drives the downregulation of megalin.

Primary rat alveolar epithelial type II (ATII) and rat lung epithelial (RLE) cells were treated with TGF-β and intracellular distribution of megalin was analyzed. Upon TGF-β treatment a marked reduction in megalin abundance at the plasma membrane and an increased concentration of the protein in the proteasome occurred. Furthermore, the PPPSP domain of megalin, the site of GSK3-β phosphorylation was mutated to determine if phosphorylation by GSK3-β upon activation by TGF-β was required for the endocytosis of megalin. Mutations that mimicked or prevented phosphorylation of the Ser residue of the PPPSP motif affected the TGF-β-induced downregulation of megalin, suggesting that the GSK3-β-specific phosphorylation site is critically involved in the cell surface stability of the protein. Moreover, after TGF-β treatment, enhanced ubiquitination of megalin was observed, and we found that Lys 14, 23, 176 and 178 were critically required for the TGF-β-induced ubiquitination and endocytosis of the receptor. Finally, treatment of ATII cells with TGF-β significantly reduced the half-life of megalin, which was fully reversed by inhibition of the proteasome but not by inhibitors of the lysosome.

Our results show that TGF-β impairs reabsorption of excess protein from the alveolar space by impairing cell surface stability of megalin by promoting proteasomal degradation of the transporter. This newly identified signaling pathway may have a therapeutic potential in patients with ARDS.
Abstract No. 302

**Mesenchymal (stem) cell heterogeneity and plasticity in influenza virus-induced lung injury: impact on therapeutic pre-conditioning to improve antiviral and tissue-regenerative MSC properties**

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Bone marrow mesenchymal stem/stromal cells (BM-MSC) are currently tested for therapeutic efficacy in clinical trials to improve outcome of ALI/ARDS. However, the mechanisms of action within the injured lung, and the use of their phenotypic plasticity allowing to improve these effects in a context of lung injury by *ex vivo* preconditioning strategies are not well defined. Influenza virus (IV) is a leading cause of morbidity and mortality worldwide. Once IV spreads to the lower respiratory tract, viral pneumonia results in severe injury of the alveolar compartment. We isolated BM-MSC (CD45/Ter119negSca-1highPDGFRα⁺) by FACS-sorting to test their plasticity and capacity to improve outcome after IV-ALI *in vivo*. Wild-type mice were infected with PR8-H1N1, and BM-MSC were applied intra-tracheally 3 days post-infection. Our results show that BM-MSC application causes a strong decrease in IV load and an epithelial-protective and proliferative response within epithelial stem cell niches, decreasing alveolar injury. Interestingly, BM-MSC were capable to respond by an injury-specific reprogramming that induced an anti-viral state in infected alveolar epithelial cells involving type I interferon signalling. An *in vitro* lung organoid model derived from endogenous lung bronchioalveolar stem cells (BASC) furthermore revealed that different heterogenic populations of BM- and lung-resident (r)MSC can support lung outgrowth and cellular differentiation, however, to different extent. BM-MSC acted in an epithelial-protective and anti-viral manner *in vivo* but they do not fully support lung organoid branching morphogenesis and epithelial differentiation, whereas rMSC strongly support those processes. Single-cell RNA-seq is now being employed to evaluate the transcriptomic diversity of murine rMSC and BM-MSC, to understand how rMSC drive lung regeneration within the BASC niche and which rMSC populations are involved. The ultimate aim is to design strategies involving preconditioning of BM-MSC, to further enhance their beneficial effects, to drive stem cell-mediated regeneration of the lung after pathogen-induced lung injury.
Abstract No. 303

**History and Research Activities of CAPNETZ STIFTUNG within the German Center of Lung Research**

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The competence network CAPNETZ was part of the action plan “Kompetenznetze in der Medizin” supported by the German Ministry of Education and Research (BMBF). The grant comprised 13.8 million Euros with a duration of ten years, until 2011. One important goal using the grant was to develop CAPNETZ into a financially self-supporting structure. For this reason the foundation CAPNETZ STIFTUNG was founded in 2007. The initial co-founders are the Charité-Universitätsmedizin Berlin, the Hannover Medical School and the University Ulm. CAPNETZ’s purpose is supporting scientific studies on CAP and other acute lower respiratory tract infections. Since 2013 the CAPNETZ STIFTUNG has been an associated partner of the DZL. In this framework the foundation continues its originally established observational CAPNETZ study. 12,000 patients are currently included in the study with information to demography, physical examination, medical history, hospitalisation, pathogen detection and clinical status up to 180 days. Different bio-samples are collected at the Hannover Unified Biobank. Since 2017 the data base has been extended with regard to actual scientific questions in pneumonia research. Following that, documentation of comorbid diseases and analysis of their role as a risk factor, investigation of the CAP influence on the course of chronic underlying diseases, investigation of HIV as a risk factor, and information about pathogens and pathogen-host interaction are included. Further activities within the DZL are the support of building up the pediatric cohort (pedCAPNETZ) and the Non-CF Bronchiectasis register PROGNOSIS in cooperation with the Hannover Medical School. Using the CAPNETZ study infrastructure such as the central office located in Hannover, the multi-study-database, the centralized biobank and the comprehensive study side network these projects were implemented successfully and will provide scientific results based on their specific research questions in the near future.
Abstract No. 304

**Localisation of Chitinase 3-like 1 in post-viral bacterial pneumonia**

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The human lung epithelium is exposed to various pathogens including the gram-positive bacterium *Streptococcus pneumoniae* and Influenza A virus. Co-infections of virus and bacteria can lead to exacerbated disease and fatal outcome as a consequence of synergism of virus and bacteria on a multifactorial basis. The epithelial cell layer functions as a first barrier of defence in the lung and responds by instructing the immune system to fight pneumonia causing pathogens. Studies have shown that the highly conserved secretion glycoprotein Chitinase 3-like-1 (CHI3L1) is involved in bacterial clearance after *S. pneumoniae* infection. Influenza-mediated alterations of CHI3L1 might provoke the increased fatality of secondary bacterial pneumonia. In this study, we investigated the expression and localisation of CHI3L1 that is crucial for bacterial killing in post-viral bacterial infections of air-liquid interface cultured primary human epithelial cells. Interestingly, Influenza alters strongly the localisation and secretion of CHI3L1 leading to a growth advantage of *S. pneumoniae*. This work for the first time observed the importance of CHI3L1 in the defence against *S. pneumoniae* in bronchial epithelial cells. It showed that Influenza mediates re-localisation of Chi3l1 which is needed to kill bacteria. This newly established co-infection model additionally functions as the basis for future infection analyses and therapeutic strategies important to achieve sustained success in fighting pneumonia.
Abstract No. 305

Microbial etiology in the CAPNETZ cohort- review of more than a decade

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Background: Detailed knowledge about the microbial etiology is the basis for empirical treatment of patients with community-acquired pneumonia (CAP) and has been prospectively assessed since 2003 within the CAPNETZ-cohort. Objectives: To determine the most relevant pathogens for CAP in Germany considering changes in microbial sampling and patient characteristics over time. Methods: Patients with confirmed pulmonary infiltrates and at least one clinical sign of lung infection were prospectively recruited from clinical centers in the CAPNETZ cohort from 2003 until 2016. For characterization of the microbial etiology of CAP only patients with complete diagnostic assessment, including urine Pneumococcal- and Legionella-antigen testing, blood cultures and bacterial cultivation of at least one respiratory sample were analyzed. Results: In total, 11.529 patients were included, on average 60.6 years old and mostly male (56.3% vs. 43.7%). Comorbidities were frequently present and did not differ between the groups. Complete diagnostics were obtained from 4.304 patients with a total of 904 detected pathogens, but frequencies of patients obtaining complete diagnostics significantly decreased over the last years. Patients obtaining complete diagnostic presented with a slightly lower CURB-65 score (1.1±1.0 vs. 1.3±1.1, p≤0.001). For both, short-term (30 days) and long-term (180 days) mortality CAP patients without diagnostics exhibited a higher mortality risk of 4.3% vs. 2.6%, and 8.4% vs. 5.6% (p≤0.001). Most prominent changes in relative pathogen distribution observed over time were a decrease of S. pneumoniae from 60% in 2003 to 40.9% in 2016 (p=0.023), and an increase of H. influenzae from 18% to 22.7% (p=0.012), respectively. Conclusion: Continuous surveillance of bacterial etiology in CAP is a pre-requisite for monitoring changes over time and adjusting treatment guidelines for more severely infected patients. This is challenged by the current practice in many hospital settings.
Abstract No. 306

**Structural and functional changes of alveolar type II cells with age and acute lung injury**

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With advancing age, the lung becomes more susceptible to injury. Alveolar type II (ATII) cells have a crucial function in lung injury and repair. However, little is known about age-related changes of ATII cells and potential impacts on acute lung injury (ALI). Here, we tested the hypothesis that ATII cell structure, surfactant protein expression and senescence differs with age and ALI.

Young (12 week) and old (18 month) mice were intranasally dosed with 2.5 mg lipopolysaccharide/kg body weight or saline. The lungs were excised 24 h later and embedded for histopathology or processed for western blot and gene expression analysis. Using stereology, the number and volume of ATII cells and their ultra-structural composition, including surfactant storing lamellar bodies, were estimated. Additionally, the expression of surfactant proteins and senescence markers was analyzed.

ALI was more severe in old compared with young mice. Expression of stress-associated senescence marker p16 was significantly upregulated with ALI in both age groups, but more pronounced in old mice. The lungs of old animals were approximately 35% larger, with a higher total number, but a lower density of ATII cells. With ALI, the number of ATII cells significantly declined by 26% in old mice, but did not change in young animals. Ultra-structural quantification indicated no alterations in volume fraction of lamellar bodies with age or ALI, however, gene expression of surfactant proteins (SP-A, B, C, D) was, with or without injury, significantly lower in lungs of old mice.

The results show that numerical density of ATII cells and expression of surfactant proteins declines with age. ATII cells of old lungs are also more susceptible to injury. These findings support the hypothesis that ATII cell function is impaired with age and thereby contributes to the worse pathology of ALI and impaired regeneration after injury in elderly individuals.
Abstract No. 307

**MiR-154 controls alveologenesis in lung development via TGF-ß signaling**

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**Objective:** MiR-154 is abundant in neonatal lungs and has been associated with several lung diseases (e.g. IPF). Experimental evidence for a direct effect of miR-154 on lung development is still lacking.

**Results:**

First, the expression of miR-154 from E10.5-P2 was analyzed by RT-qPCR and FISH. MiR-154 expression increased dynamically from E10.5 and peaks at P2. Attenuation of miR-154 in WT embryonic lung (CD1) harvested at E11.5 and treated with morpholino vs. control reveals a significant decreased in lung branching after 48h of culture. Prenatal overexpression of miR-154 from E7.5-E18.5 in CCSPrtTA/rtTA;tet(O)miR-154/+ mice (lung epithelial specific overexpression) and analysis of the lung structure by using alveolar morphometry shows increased mean linear intercept (MLI) and alveolar airspace but decrease in septal thickness indicating alveolarization defects. RT-qPCR reveals a decrease in Fgf10 signaling as well as markers for alveolar myofibroblast but increase in epithelial marker EpCAM. In contrast, postnatal overexpression of miR-154 by using CCSPrtTA/rtTA;tet(O)miR-154/+ from P0-P16.5 leads to increased Fgf and Tgf-ß signaling in the mutant group as well as increased MLI without changes in airspace and septal thickness. Interestingly, pull-down assay reveals caveolin 1 (Cav1), which is responsible for Tgf-ß/Tgf-ßR degradation, as a target of miR-154.

**Conclusions:**

MiR-154 plays a crucial role in alveologenesis – possibly involving Tgf-ß signaling.
Abstract No. 308

The PROGRESS Study - Operationalization of Disease Severity and Prediction of Outcome using the SOFA Score

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Purpose: CAP (Community acquired pneumonia) is frequent, with a high burden on health care systems. Optimized severity assessment and risk stratification may aid management of CAP patients and reduce mortality. Development of predictive biomarkers and new therapeutic concepts require a valid, reproducible, and quantitative assessment of CAP severity and better risk stratification.

Patients and methods: Using time series data of the first 1,532 patients enrolled in the multi-center PROGRESS study, we compared existing CAP severity scores for operationalization of disease severity and for their performance in correctly predicting a subsequent severe course of CAP (death or need for intensive care with at least one of the following: substantial respiratory support, treatment with catecholamines, or dialysis). Considered scores were CRB-65, CURB-65, PSI, SIRS-Score, SOFA, qSOFA, Halm criteria, SCAP, SMART-COP, and the two laboratory parameters CRP and PCT. Comparisons were performed by receiver operating characteristics or precision recall analysis using R. Patients enrolled in the PROGRESS study were younger than the overall German population of hospitalized CAP patients (median of 59 vs. 73 years) with lower in-hospital mortality (2.3% vs. 13.9%).

Results: SOFA significantly outperformed all considered alternatives in identifying patients with a severe state of disease at the day of enrolment (AUC = 0.948) and thereafter. Age, sex, and pack-years significantly contributed to higher SOFA values whereas antibiosis before hospitalization predicted lower SOFA. 28-day mortality was predicted with AUCs > 80% (SOFA, PSI, SCAP, CURB-65, SMART-COP, decreasing AUC). Need for later intensive care treatment was predicted with highest AUC below 79% (SOFA, SCAP, SMART-COP, CURB-65, Halm, PSI, decreasing AUC).

Conclusion: SOFA and closely related scores well suited for operationalization of CAP severity. SOFA is predictive for 28-day mortality or need for ICU admission, but with limited specificity. Biomarkers further improving sensitivity and specificity would be highly desirable for clinical decision-making.
Abstract No. 309

**Integrin αE (CD103) is dispensable for pneumococcal colonization-induced adaptive immune responses and protection against invasive pneumococcal disease in mice**

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Nasopharyngeal colonization with Streptococcus pneumoniae is known to mount protective adaptive immune responses in rodents and humans. However, the cellular response of the nasopharyngeal compartment to pneumococcal colonization and its importance for the ensuing adaptive immune response is only partially defined. The study aims to evaluate the impact of CD103 deficiency on nasopharyngeal colonization-induced adaptive immunity in mice. We show that nasopharyngeal colonization with S. pneumoniae triggers substantial expansion of both CD103pos DCs and CD103pos T cells in the nasopharynx, NALT and CLN of mice. However, nasopharyngeal de-colonization and pneumococcus-specific antibody responses as well as protection against invasive pneumococcal disease (IPD) were similar between WT and CD103 KO mice. Similar results were obtained in WT and BATF3 KO mice specifically lacking CD103+ DCs. In summary, the data show that CD103 is dispensable for pneumococcal colonization-induced adaptive immune responses in mice.
Abstract No. 310

Lung injury and repair in vitro

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Objectives: Lung epithelium repair after injury is a complex process involving the coordination of multiple cell types. An accurate description of the tissue’s reaction to injury and the role of specific cell populations requires a close follow-up with precise imaging of this process. We employed our established tissue culture system to image the wound response after injury caused by sectioning. We traced specific cell types by combining this system with an in vitro tamoxifen induction of Cre-loxP recombination to label and trace specific cell types.

Results: Lungs of Cre mice carrying fluorescent reporters were harvested and sectioned using a vibratome. Precision-cut lung slices of 200μm were cultured for up to 14 days. Alveolar (ATII) or bronchiolar (club) cells were labeled after induction with tamoxifen. Confocal microscopy, live imaging, immunofluorescence staining and image analysis of the reconstructed 3D alveolar and bronchial architecture was performed. We were able to quantify proliferation and stain with markers of alveolar or bronchial markers.

Conclusions: We were able to demonstrate a lung epithelial wound response to the injury caused by the vibratome blade. This response was limited to the surface of the slices where the injury occurred. Labelled cells proliferated and expanded/repaired the wounded epithelium.
Abstract No. 311

High CO2 levels induce downregulation of ENaC by increasing phosphorylation and polyubiquitination of ENaC β-subunit in human lung epithelial cells

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Elevated levels of CO2 in the blood (hypercapnia) are often observed in patients with acute respiratory distress syndrome (ARDS) and are a result of poor alveolar gas exchange. Impaired alveolar fluid clearance (AFC) is detected in most patients with ARDS and these patients require lung-protective mechanical ventilation with low tidal volumes, which however often leads to further accumulation of CO2. The vectorial Na+ transport across the alveolar epithelium, driven by the apically-expressed epithelial sodium channel (ENaC) represents the primary mechanism for AFC. Our results demonstrate that short-term exposure to hypercapnia (pCO2~120 mmHg, pH 7.4 for 30 min) rapidly alter ENaC cell surface stability in human lung epithelial cells decreasing both total and amiloride-sensitive Na+ transport. The hypercapnia-induced increase in α/β-ENaC complex endocytosis was driven by increased polyubiquitination of β-ENaC mediated by the E3 ubiquitin ligase Nedd4-2. Both events were prevented by inhibition of AMP-activated protein kinase (AMPK)-driven and JNK1/2-dependent Nedd4-2 phosphorylation at the Thr899 residue. Substitution of Thr899 by Ala within the catalytic domain of Nedd4-2 rescued the abundance of the channel at the cell surface upon hypercapnic exposure. Moreover, in the hypercapnic signaling cascade, enhanced ENaC ubiquitination and cell surface retrieval were strongly dependent on β-ENaC phosphorylation at Thr615 mediated by the extracellular signal regulated kinase (ERK)1/2 (upstream kinase of AMPK). Transfection of cells with a β-ENaC mutant lacking Thr615 rescued cell surface density of the α/β-ENaC complex upon hypercapnic exposure. Thus, this novel hypercapnia-induced signaling pathway increases β-ENaC/Nedd4-2 interaction promoting polyubiquitination of β-ENaC and endocytosis of the α/β-ENaC complex, decreasing ENaC-mediated Na+ transport, which may contribute to retention of lung edema in hypercapnic patients with ARDS.
Abstract No. 312

RNA-mediated regulatory networks in Legionella pneumophila pneumonia

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Legionella pneumophila (L.p.) is a gram-negative bacterium and is a common cause of severe community-acquired pneumonia. In humans, L.p. replicates within alveolar macrophages. Thereby, L.p. manipulates the host to promote its own replication. The transcriptional profile of L.p. during the course of infection in human macrophages is yet to be investigated. Additionally, the altered gene expression of Legionella infected macrophages has only been performed in a mixed population of infected and non-infected cells. This study aims at identifying gene expression changes during the course of infection of both, host and pathogen. To elucidate the transcriptional profile of both simultaneously, we used a dual-RNA-Seq approach.

THP-1 cells were infected using the GFP-expressing strain L.p. Corby for 8 and 16 h, with a multiplicity of infection (MOI) of 10. To separate infected cells (gfp+) from the non-invaded bystander cells (gfp-), FACS-Sort was performed. After RNA isolation, rRNA was depleted and libraries for sequencing were prepared. Sequencing reads were mapped to the genomes of human and L.p., respectively. An average of 11205312 and 4594488 reads were mapped per sample to the human and Legionella genomes respectively. Differential gene expression analysis was performed using DESeq2 resulting 4914 differentially expressed human genes (across multiple conditions) and 2720 Legionella genes (across 2 time points). The DESeq analysis of the separated RNA fractions from host cells revealed differentially expressed miRNAs (145 genes), mRNAs (3,504 genes) and lincRNAs (495 genes). We found 1,152 differentially expressed genes, which were exclusively significantly regulated in the invaded cells. The heatmap of differentially expressed host mRNAs shows promising gene clusters which indicate differential gene regulation at different times and conditions.

In summary, the results provide new insights into the infection process and will help to understand the complex interplay between Legionella and their human host to create a complex RNA interaction network.
Hypocapnia Aggravates Hypoxic Inhibition of ENaC in Rat Primary Alveolar Epithelial Cells

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RATIONALE: Hypoxia inhibits Na-reabsorption across alveolar epithelium by decreasing the activity of epithelial Na channels (ENaC) and Na/K-ATPase, caused by decreased mRNA and protein expression. Also alveolar Na-dependent water reabsorption was inhibited in anesthetized hypoxic rats. It has therefore been postulated that inhibition of alveolar reabsorption might contribute to the occurrence of alveolar edema in hypoxia. In vivo hypoxia is associated with hyperventilation-induced hypocapnia. However, effects of hypocapnia had never been studied in cultured cells. METHODS: Rat primary alveolar epithelial cells were exposed to normoxia (N) and hypoxia (1.5% O2; H) in normocapnia and hypocapnia (5% and 3% CO₂, resp.) for 24 hours (N₅, N₃, H₅, H₃); Na-transport was measured in Ussing chambers. RESULTS: Hypoxia decreased ENaC (-80%) and Na/K-ATPase (-42%) activity measured in normocapnia with bicarbonate as the sole buffer (N₅ vs. H₅). Interestingly, ENaC activity was even further decreased by hypocapnia (-60%; H₃ vs. H₅), whereas Na/K-ATPase was not. Keeping extracellular pH stable, independent of changes due to hypocapnia did not affect this result. Changes in Na-transport were not prevented by carbonic anhydrase inhibition. Also inhibition of protein kinase C, ERK, and AMPK, which seem involved in inhibition of alveolar transport by hypercapnia (Vadasz & Snajder, Front Immunol 2017), did not prevent the additional inhibition of ENaC, nor the hypoxic inhibition of ENaC and Na/K-ATPase. There was a decrease in α-, β-, and γ-ENaC mRNA expression by ~70% by hypoxia, and a decrease in αENaC protein abundance in the apical membrane (measured by membrane biotinylation) as well as in the intracellular compartment by ~50% in hypoxia. However, none of these parameters was affected by hypocapnia, neither in normoxia nor in hypoxia. CONCLUSIONS: These results indicate that hypocapnia enhances hypoxic inhibition of alveolar Na-reabsorption, where the additional ENaC-inhibition was independent of alkalosis, PKC and AMPK.
Abstract No. 314

Data from the PROGRESS Study - Blood Gene Expression Patterns predict severe Outcome of Community Acquired Pneumonia

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Purpose: CAP (community acquired pneumonia) is a frequent infectious disease with severe manifestations, associated suffering, and high costs to health care systems. Improvement of risk stratification by blood gene expression biomarkers predicting severe outcomes may support clinical decision-making and reduce mortality of CAP patients.

Patients and methods: Within the PROGRESS study, clinical development of patients hospitalized with CAP was followed for 28 days, including daily assessment of clinical parameters within the first five days and sampling of peripheral blood for the first four days. Disease severity was operationalized by SOFA score. Death and requirement of intensive care with at least one of the following: substantial respiratory support, treatment with catecholamines, or dialysis were considered severe endpoints. Time series of genome-wide gene-expression measurements of whole blood samples of 394 patients were used to analyse their potential for prediction of time courses and endpoints. Analyses were accompanied by comprehensive pathway activation analyses and comparisons with gene-expression patterns observed in related sepsis studies.

Results: About 50 genes were strongly associated with a future severe course of disease, >5000 genes were associated with current CAP severity and >250 genes with current severe endpoints. With increasing CAP severity, pathway analysis showed increased inactivation of many immune-related pathways, especially related to T-cells. Previously proposed expression signatures correlate well with current, but poorly predict future CAP severity. We created a new classifier to predict future severe endpoints and evaluated its performance compared to prediction based on the SOFA score.

Conclusion: Increasing CAP severity is accompanied by substantial regulation of gene expression and immune-related pathways. Several transcripts may be the basis for new biomarkers helping to improve diagnosis, risk stratification, therapy decisions, and outcome for patients with CAP.
Abstract No. 315

Elevated CO2 levels decrease Na,K-ATPase cell surface abundance by causing ER retention of the transporter

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Normal protein folding in the endoplasmic reticulum (ER) is important to maintain cellular homeostasis. Impaired ER maturation of the Na,K-ATPase, a key molecule involved in the regulation of sodium transport and alveolar fluid clearance, may decrease protein abundance of the transporter at the plasma membrane and could be deleterious in lung diseases associated with alveolar edema. Here, we investigate how hypercapnia, which is often observed in patients with acute respiratory distress syndrome, affects ER folding of the Na,K ATPase. Exposing human alveolar epithelial A549 cells to elevated CO2 concentrations (up to 120 mmHg, pH 7.4) led to a significant reduction of the Na,K ATPase α- and β subunit at the plasma membrane. Furthermore, hypercapnia induced retention of the Na,K ATPase β subunit in the ER in a time- and dose-dependent manner. Importantly, the ER-retained Na,K ATPase β subunit was not assembled with the α-subunit, suggesting that hypercapnia prevents normal protein folding of the enzyme. Of note, elevated CO2 levels did not increase protein oxidation. Treatment with α-ketoglutaric acid or 1,2-bis(2-aminophenoxy) ethane-N,N,N′,N′-tetraacetic acid–acetoxymethyl ester (BAPTA-AM) decreased the amount of ER-retained Na,K ATPase β subunit, suggesting that ATP and Ca2+ dependent mechanisms were potentially involved in the retention of the enzyme. Additionally, hypercapnia activated unfolded protein response (UPR) and increased phosphorylation of eukaryotic translation initiation factor 2α (eIF2α) by PKR-like endoplasmic reticulum kinase (PERK). Moreover, siRNA knockdown of either eIF2α or PERK increased the ER amount of the Na,K ATPase β subunit, suggesting activation of an adaptive mechanism in the ER. Our results suggest that hypercapnia decreases plasma membrane abundance of the Na,K ATPase by promoting retention of its β subunit in the ER. Elevated CO2 levels activate adaptive UPR by increasing phosphorylation of eIF2α by PERK.
Abstract No. 316

**Tubulin-dependent delocalization of cellular Na,K-ATPase during influenza A virus infection.**

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One of the complications induced by influenza A virus (IAV) infection is the acute respiratory distress syndrome (ARDS), which is characterized by accumulation of fluid within the alveolar lumen. Clearance of lung edema depends on presence of an osmolytic gradient created by several membrane ion-channels, including Na,K-ATPase (NKA). Previously, it was shown that IAV-infection of primary human and murine alveolar epithelial cells (i) decreases NKA amount on the basolateral membrane of neighbouring, non-infected cells and (ii) affects its distribution within the plasma membrane of infected cells (Peteranderl C. et al., JCI, 2016). Here, we tried to elucidate the molecular mechanism underlying apical distribution of NKA in infected cells. Translocation of NKA from basolateral to the apical cell membrane during IAV-infection could be confirmed using a permanent culture of human bronchiolar epithelial cells (Calu3). The NKA relocalization was investigated by two independent methods: (i) biotynilation of apical cell membrane proteins and quantification by "on cell western blot" and (ii) qualitative analysis via immunofluorescence. After infection of the Calu3 cells with different IAV we observed that the NKA translocation did not depend on subtype of the IAV or the viral replication kinetics. Furthermore, an application of different chemical inhibitors, which have a direct influence on the cell cytoskeleton or on cytoskeleton regulatory molecules revealed that NKA translocation is at least in part dependent on the integrity of the tubulin network. In addition, treatment of infected cells with an inhibitor of Rho-kinase not only prevents the apical translocation of NKA, but also improves a vectorial water transport through Calu3 cell monolayer. The source of NKA protein, which appears on apical cell membrane after IAV infection seems to be the intracellular depot of NKA, since the transport inhibition of newly synthesized NKA does not prevent its apical localization.
Depletion of the resident alveolar macrophage pool upon influenza virus infection is a death ligand-dependent apoptotic event

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RATIONALE: Resident alveolar macrophage (rAM) depletion is a major step towards disease progression in influenza virus (IV)-induced lung injury and acute respiratory stress syndrome (ARDS). Therefore, we aim to elucidate the time course, cellular crosstalk and underlying molecular pathways, as improving rAM survival poses a promising therapeutic target in order to ameliorate disease severity.

METHODS: C57BL/6 wild-type mice were infected intratracheally with influenza virus (IV) PR/8. Bronchoalveolar lavage (BAL) was harvested at different time points for flow cytometry (FACS) analysis. Additionally, FACS was performed on rAM after ex vivo infection and treatment with BAL from infected mice. Apoptosis-inducing ligands and rAM death pathways were analyzed on gene expression level in flow-sorted rAM as well as in primary isolated rAM after ex vivo infection.

RESULTS: FACS analysis revealed that significant rAM depletion begins on day 3 post-IV infection with rAM numbers reaching their lowest level on day 7. Although the majority of rAM was subjected to apoptotic death, the percentage of directly infected rAM by influenza virus was discrepantly low. In vitro treatment of naive rAM with day 7 BAL had a more prominent apoptotic effect than direct PR8 infection 24h after treatment. Gene expression analysis of flow-sorted rAM revealed intrinsic apoptosis to be the main death-driving pathway, as indicated by significant upregulation of factors such as Fas ligand, Caspase-9 and Bax (Bcl-2 associated X protein). Further investigation of potential apoptosis triggering ligands showed a significant upregulation of various TNFSF members, such as TNFSF 10, 14 and 15.

CONCLUSIONS: IV infection of wild-type mice leads to rapid rAM depletion by ligand-driven intrinsic apoptosis. Dissecting the specific cellular interactions and blocking the underlying molecular signaling events is expected to enable us to improve rAM survival, and ultimately provide a novel therapeutic strategy for a better outcome in IV-induced pneumonia and ARDS.
Stroke causes structural and functional changes of the murine tracheal epithelium

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Objective: Pneumonia is the most relevant complication within the first three months after stroke. It has a tremendous negative impact on the neurological outcome and often causes death of stroke patients. We hypothesized that stroke induces structural changes of the tracheal epithelium resulting in impaired mucociliary clearance, thereby preparing the ground for the development of pneumonia. Methods: The middle cerebral artery occlusion (MCAo) model (60 min artery occlusion; analysis of the trachea 24 h or 49 days after stroke) in mice was used to examine structural and functional changes of the respiratory epithelium. Ciliated and secretory cells were identified by immunohistochemistry employing antibodies raised against β-tubulin and surfactant protein D (SP-D), respectively. Ciliary beat frequency of single cells and cilia-driven transport of particles on the surface of explanted tracheae were measured. Results: In cross-sectioned tracheae of mice killed 24 h after MCAo, the number of ciliated cells was decreased and that of secretory cells was increased compared to sham operated animals. The degree of these changes differed considerably between individual animals. In whole mount tracheae, we observed in some samples a minor reduction in the number of ciliated cells whereas in other samples this cells type was drastically reduced. The degree of remodelling was not correlated to the Bederson score or to infarct size. After 49 days the number of ciliated cells was roughly equal in MCAo and sham-operated mice. In tracheae isolated 24 h after stroke, particle transport speed was significantly decreased as compared to naive and sham-operated controls. Notably, at the level of single cells, ciliary beat frequency was unchanged. Conclusion: Stroke causes rapid changes in the tracheal epithelium which may result in insufficient mucociliary clearance favoring the development of pneumonia.
Abstract No. 319

A Bacterial Signal Peptide Increases Mucociliary Clearance in Explanted Mouse Trachea by Stimulating Chemosensory Cells and Paracrine Cholinergic Signaling

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Objective: Mucociliary clearance (MC) is a major innate defense mechanism that removes pathogens from the airways. Bacterial quorum sensing molecules stimulate MC by activating the bitter taste signaling cascade. Bacterial formylated signal peptides, activate innate immune cells via formyl peptide receptors (FPR). We here investigated whether this novel class of agonists also influences mucociliary clearance.

Methods: Cilia-driven particle transport speed (PTS), a readout for MC, was studied in wildtype (C57Bl6) and in mice lacking a functional FPR3 (FVB/NCrl), components of the taste transduction cascade (TRPM5 (transient receptor potential channel 5); ITPR3 (inositol 1,4,5-triphosphate receptor 3); PLCβ2 (phospholipase Cβ2)) or solitary chemosensory cells (TRPM5-DTA). The transcriptome of single tracheal ciliated and brush cells (cholinergic chemosensory epithelial cell type) was analyzed by deep sequencing.

Results: The N-formylated bacterial signal peptide FL185 present in various pathogens, e.g. E. coli and Salmonella typhimurium, increased PTS from 45±2 to 73±3 µm/s (mean±SEM; p<0.0001; n=18). Deep sequencing showed FPR expression in both ciliated and brush cells and presence of TRPM5, PLCβ2, ITPR3 and several chemoreceptors including bitter receptors in brush cells. FPR1 and FPR2 inhibitors (cyclosporine H (1 µM) and t-BOC2 (10 µM)) did not reduce the effect. It was also conserved in FVB/NCrl mice, but significantly reduced in TRPM5-, PLCβ2- and in ITPR3-deficient and TRPM5-DTA mice. Atropine (1 µM) and 4-DAMP (1 µM), muscarinic receptor antagonists, significantly diminished the effect of FL185. Nicotinic antagonists (mecamylamine (100 µM)) and antagonists of voltage gated sodium channels (tetrodotoxin (1 µM) had no effect.

Conclusion: A bacterial signal peptide stimulates MC independently of FPR. Instead, this effect involves typical taste transduction elements, tracheal brush cells, and subsequent cholinergic signaling to ciliated cells. Thus, detection of a defined set of bacterial signal peptides by brush cells provides a novel defense mechanism against bacteria.
Abstract No. 320

Bordetella pseudohinzii targets cilia and impairs tracheal cilia-driven transport in community-acquired infection in mice

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Objective: Characterization of the pathogenic potential of a newly described Bordetella strain which colonizes the respiratory tract of laboratory mice.

Methods:
Bacterial genome was analyzed using Illumina´s NextSeq 500 next generation sequencing system. Tracheal particle transport speed (PTS) and ciliary beat frequency (CBF) were analyzed with a high speed camera and by tracking dynabeads. Histopathological changes of the airways were analyzed by H&E staining. Cultured tracheal rings were infected with isolated Bordetella pseudohinzii for 4 or 24 h and attachment of bacteria was visualized by electron microscopy (SEM and TEM).

Results: Four bacterial isolates from lung and trachea of SPF-kept mice were identified as Bordetella pseudohinzii by genomic sequencing, presenting as rod-shaped coccobacilli with peritrichous flagellae in electron microscopy. Community-acquired infection with B. pseudohinzii lead to decreased number of tracheal ciliated cells (42.42 n=8 uninfected to 30.61.7% infected; n=5; meanSEM), reduced CBF (18.21 0.60 to 12.121.48 Hz n=7) and PTS (baseline: 60.72.76 n=19 to 24.512.79 µm/s; n=11). B. pseudohinzii-positive animals showed no clinical signs of infection, but neutrophils in BAL were highly increased (0.240.04 to 14.394.6%; n=6). Histopathological analysis revealed tracheitis, interstitial pneumonia, and formation of tertiary lymphoid follicles (bronchus-associated lymphoid tissue = BALT) along the main and secondary bronchi. In in vitro-infected tracheal rings, B. pseudohinzii attached to ciliated cells, formed biofilms and damaged the epithelium. PTS and CBF were significantly reduced after incubation for 4 h with 1.6x105 CFU.

Conclusion: Colonization of mice with B. pseudohinzii leads to BAL neutrophilia, inflammation of the respiratory tract and impaired mucociliary clearance due to reduction and damage of ciliated cells. B. pseudohinzii may represent a novel mouse specific model organism to study closely related human lung pathogens like Bordetella hinzii and pertussis.
Abstract No. 321

Rate and Predictors of bacteremia in afebrile community-acquired pneumonia (CAP)

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Background: Depending on the underlying pathogen, bacteremia is a grave complication of serious prognostic relevance in community-acquired pneumonia (CAP), and its timely recognition is pivotal in the selection of targeted therapy. “Fever” has been traditionally used as a trigger for blood culture (BC) sampling.

Objectives: This study aims to describe i) the proportion of patients with CAP and bacteremia in the absence of fever, and ii) the clinical characteristics which can predict the necessity of BC in afebrile CAP patients.

Methods: Bacteremia rates were determined in 4349 adult CAP patients enrolled by the German prospective cohort study CAPNETZ and were stratified by febricty at first patient contact. To identify independent predictors of bacteremia in afebrile patients, demographic data, co-morbidities, antibiotic pretreatment and clinical signs were retrospectively compared between afebrile patients with and without bacteremia by multivariate logistic regression analysis.

Results: Bacteremic pneumonia with a CAP-specific pathogen was present in 209 of 2854 (7.3%) febrile patients and 52 of 1495 (3.5%) afebrile patients. Age distribution and inflammation parameters did not significantly differ (p>0.05) in bacteremic CAP patient with and without fever. Antibiotic pretreatment decreased the rate of bacteremia with a CAP-specific pathogen (OR 0.35, p<0.001). In logistic regression model, we identified need of oxygen administration (AOR 4.75, 95% confidence interval [CI], 1.41-15.99), chronic kidney disease (AOR 2.23, 95% CI, 1.10-4.95) and mental confusion (AOR 3.17, 95%CI 1.34-7.52) as independent predictors of bacteremia in afebrile CAP patients.

Conclusion: As 20% of BC with CAP-specific isolates originated from non-febrile patients, the relevance of febricty as indicator of BC necessity merits reconsideration. Need of oxygen administration, mental confusion, and renal co-morbidity appear to independently predict bacteremia in non-febrile CAP patients.
Abstract No. 322

C-reactive protein – a safeguard against trauma-induced sterile inflammation

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Introduction: Blood levels of the acute phase reactant C-reactive protein (CRP) are frequently measured as a sensitive clinical marker for inflammation. The biological functions of this phosphocholine (PC)-binding pentraxin are still controversial. Recently, we discovered that PC inhibits the ATP-mediated release of IL-1β from monocytes via unconventional nicotinic acetylcholine receptors (nAChR). The clinical interest in mechanisms controlling IL-1β is outstanding, as IL-1β is a pathogenetic factor of life-threatening systemic inflammatory diseases like systemic inflammatory response syndrome. Here, we hypothesized that native CRP/PC complexes control ATP-induced IL-1β release.

Methods: In vitro experiments were performed on lipopolysaccharide-primed human monocytic U937 cells and freshly isolated peripheral blood mononuclear cells (PBMC) from healthy human donors stimulated with ATP in the presence and absence of CRP. IL-1β was measured in cell supernatants by ELISA. A panel of nAChR antagonists, siRNA technology and electrophysiology measurements on U937 cells were used to test if CRP signals via nAChR. Activation of caspase-1, maturation of IL-1β and pyroptosome formation were investigated in PBMC by Western blotting and immunocytochemistry. To test if high CRP levels inhibit trauma-induced IL-1β release in vivo we performed a small prospective study on patients suffering from multiple traumata.

Results: CRP efficiently inhibited ATP-induced IL-1β secretion (IC50 of 5 µg/ml). The inhibitory effect of CRP relies on the calcium-dependent association with a small molecule, presumably PC and was prevented by antagonists for nAChR. In patch-clamp measurements on U937 cells, CRP abrogated ATP-induced P2X7 receptor activation without inducing ion currents at classical nAChR. In PBMC stimulated with ATP, CRP impaired the activation of caspase-1, maturation of IL-1β and pyroptosome formation. Furthermore, in blood samples of multiple-trauma patients IL-1β plasma levels negatively correlated with CRP values.

Conclusions: CRP is an unconventional nAChR agonist that potently inhibits ATP-induced inflammasome activation and might protect against trauma-associated sterile inflammation.
Abstract No. 323

Immunophilin- and MAPK-associated pathways as therapeutic targets in severe human MERS-CoV infection

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The middle east respiratory syndrome Coronavirus (MERS-CoV) emerged for the first time in 2012 in Saudi Arabia and until December 2017, over 730 people died from MERS-CoV infection. It is a causative agent of acute lung injury and ARDS, and drugs providing protection against emerging MERS-CoV are lacking. An interesting candidate for drug therapy was found to be Cyclosporin A (CsA) as previous studies showed that CsA decreases MERS-CoV replication but so far the mechanism is unclear. To mechanistically address its potential use as antiviral against coronaviruses, we specifically blocked single hubs of host pathways affected by CsA within the cell, and presumably relevant for MERS-CoV replication. Using airway epithelial cells (Calu-3), and primary human alveolar epithelial cells, we found that the inhibition of NFAT, JNK and p38 significantly reduced virus release of MERS-CoV, suggesting that immunophilin/MAPK targeting by CsA can inhibit MERS-CoV replication in human lungs. As no rodent model for MERS-CoV closely modeling human lung pathology existed due to lack of expression of the receptor relevant for viral entry (human DPP4), we developed a novel mouse model using adenoviral transfer and expression of the human DPP4 receptor in the alveolar epithelium, followed by MERS-CoV infection. Using this model, we succeeded to obtain high transduction efficacy for the MERS-CoV receptor within the alveolar epithelium, and to induce severe infection and lung injury in mice. In cooperative work between DZIF and DZL we will further analyze the effect of CsA on MERS-CoV infection by RNA-Seq in vitro and in in vivo experiments, to comprehensively understand the downstream effectors and involved transcriptional networks of immunophilin/MAPK targeting related to MERS-CoV replication, and to gain further insights into the potential use of CsA as therapeutic drug.
Abstract No. 324

Pro-viral miRNAs are enriched in BALF extracellular vesicles of patients with influenza-induced ALI

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Influenza A virus (IAV) is a major pathogen causing seasonal and pandemic respiratory infections and resulting in about 3 to 5 million cases of severe illness, and about 250,000 to 500,000 deaths worldwide. In the USA, direct and indirect costs of seasonal IVA are estimated at 10 and 16 billion USD per year, respectively. Despite worldwide vaccination programs, IAV still causes primary viral pneumonia with rapid progression to acute lung injury and fatal outcome. This acute lung injury is characterized by severe damage of alveolar epithelium, resulting in a breakdown of gas exchange. Paracrine communication between alveolar macrophages and lung epithelial cells has been shown to be critical for this IAV-induced epithelial damage. Extracellular vesicles (EVs) like exosomes have been observed in lung diseases like asthma in bronchoalveolar lavage fluid (BALF). They have been shown to contain specifically enriched microRNAs (miRNAs), and to provoke specific pathogenic effects in recipient cells. It is still unclear whether this is also the case in human influenza infection. To answer this question, we analyzed BALF from six patients with IAV H1N1-induced acute lung injury (ALI; mean age 47.8 ± 13.0 yr, 17% male) and seven healthy volunteers (mean age, 27.3 ± 5.3 yr, 42% male). We identified a significant change of abundance of several miRNAs in EVs from BALF of patients with IAV-induced ALI and – partly overlapping – from the supernatant of IVA-infected lung epithelial cells. miRNA 17-5p was upregulated both in vivo and in vitro. It downregulated antiviral transcripts such as Mx1 and facilitated IVA replication in lung epithelial cells. We show for the first time upregulation of miR-17-5p in BALF exosomes during influenza-induced acute lung injury and its hindering effect on influenza-control by host cells.
Abstract No. 325

Strategies for the Treatment of Community-Acquired Pneumonia in HIV-Positive Patients

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Background: According to the Robert Koch Institute, 84,700 people in Germany suffer from HIV infection. One-third of the affected persons is over 50 years old. In Germany, community-acquired pneumonia (CAP) is a widespread disease with more than 250,000 cases per year. Incidence and mortality increase with the age of the affected individuals. Objectives: Diagnostic and therapeutic strategies are needed to guide medical care of HIV-infected patients presenting with CAP. Methods: HIV therapists were interviewed about their diagnostic approach, risk stratification strategy and therapeutic approach to HIV-associated community-acquired pneumonia (HIV+/CAP) using a questionnaire. 56 completed questionnaires were analysed. Results: Half of the respondents reported that CAP occurred in 1 to 5% of HIV-infected individuals per year. This indicates an estimated number of up to 4200 HIV+/CAP cases per year in Germany—a much higher number than expected from the literature. 58.9% of respondents considered that the pathogenic spectrum did not differ in HIV+/CAP from non-HIV/CAP. 80.3% of respondents applied the same antibiotic regimens in HIV+/CAP as used in patients with non-HIV/CAP. Conclusion: Even though over 40% of HIV therapists agree that the pathogenic spectrum of HIV+/CAP differs from that of non-HIV/CAP, over 80% of therapists managed these patients in accordance with the S3-guidelines for non-immunocompromised CAP-patients, because specific guidelines for the treatment of HIV+/CAP are lacking. Since specific data on the aetiology and the clinical course of HIV+/CAP depending, for instance, on CD4-count and antiretroviral therapy are missing, we feel that the clinical course of HIV+/CAP should be further analysed in the context of prospective cohort studies.
Abstract No. 326

LncRNAs in human lung innate immunity

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Since the release of the ENCODE human transcriptome annotation in 2012 thousand of novel non-protein-coding gene loci have been discovered. Recent estimates suggest that the human genome contains approximately 15000-18000 long non-coding RNA (lncRNA) genes, which per definition give rise to transcripts longer than 200 nt. We have used RNA-Seq to screen for long non-coding RNAs with possible implications in human alveolar epithelial and macrophage innate immune responses to bacterial pathogens. Our data reveal dozens of immune-responsive lncRNA loci, which we are currently characterizing through lentiviral overexpression and CRISPR/Cas9 mediated gene knock-out experiments. Our preliminary results suggest major implications of lncRNAs in diverse aspects of innate inflammatory responses including myeloid cell differentiation and survival, Pattern Recognition Receptor (PRR) signaling and intracellular antimicrobial defense. This suggests that lncRNAs serve as critical regulators of human lung innate immunity to microbial pathogens.
Abstract No. 327

Plasticity and functional phenotype of bone marrow derived macrophages in influenza virus-induced lung injury and repair

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RATIONALE: Bone marrow derived macrophages (BMM) play an important role in host defense and tissue homeostasis. Macrophage polarization has been extensively studied in different disease models, but the generation of macrophage phenotypes with specific functional profiles, their particular roles during pathogen-induced acute lung injury (ALI), resolution and tissue repair have not been convincingly elucidated.

METHODS: C57BL/6 mice were infected with influenza virus PR/8 by intra-tracheal (i.t) application. BAL and lungs were harvested during the acute, resolution and repair phases of infection. The polarization profiles of different subsets of BMM were characterized by FACS during the time course of infection. Bone marrow transplantation experiments using CD45.2/1 mice were performed to demonstrate lineage-relation between recruited and resident macrophages. Adoptive transfer of BMM into the lungs of IV-infected CCR2-/- mice was performed to address their functional phenotypes. Gene expression profiles in different BMM phenotypes were screened by transcriptome analysis.

RESULTS: FACS analyses demonstrated that BMM show an M1-like phenotype in the acute phase and shift to an M2-like phenotype in the late phase of infection. BMT experiments revealed that M2-type substantially contributed to replenishment of the depleted rAM pool, indicating a high functional plasticity of BMM recruited after infection. Adoptive transfer experiments showed that transferred M1-type increased alveolar barrier dysfunction whereas M2-type preserved the rAM pool, inducing AEC proliferation and barrier repair. Transcriptome analysis showed significant up-regulation of a set of growth factors, repair mediators and pro-survival genes in M2-type, some of which were found to be mediators of the beneficial functions of M2-type BMM in vivo.

CONCLUSIONS: These data support that mediators produced by M2-type BMM contribute to replenishment and preservation of the rAM pool and improved lung barrier function. In summary, our data demonstrate high functional plasticity of BMM during IV pneumonia and highlight these cells as targets for therapeutic approaches.
Role of TRAIL in the pathogenesis of Bronchopulmonary Dysplasia (BPD)

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Objectives:

BPD is mainly caused by an inflammatory response of the immature lung to mechanical ventilation and oxygen toxicity. Members of the TNF-superfamily are known for their heterogeneous impact in different diseases. While FasL is detrimental in the situation of hyperoxic lung injury, baseline TNF-α protects the immature lung. Here, we aimed to unravel the impact of the death inducing ligand TRAIL during hyperoxic lung injury in the newborn mouse with respect to lung histology, inflammation and apoptosis and surfactant production.

Methods:

Wildtype (C57BL6) and TRAIL⁻⁻ (B6.129-tnfsf10tm1Mjs) mice were exposed to room air (21% O₂) or 85% of oxygen from P1 to P8. Analyses at P8 and P28 include survival rates, lung histology, and determination of the extent of cell death induction and of inflammatory response and SPC-production.

Results:

Lung histology at P8 showed fewer and larger alveoli in the TRAIL⁻⁻ mice. The parameters of alveolar morphometry i.e. air space percentage and mean linear intercept showed a statistically significant (p<0.05) increase in TRAIL⁻⁻ mice. However no differences were observed for the mRNA expression of IL-1β, TNF-α or TGF β at P8. Tunel staining revealed no difference in cell death induction, whereas Immunofluorescence staining of Surfactant protein C showed higher number of SPC positive cells in TRAIL⁻⁻ mice which did not attain a statistical significance.

Data at P28 will be available at the time of poster presentation.

Conclusions:

Trail⁻⁻ mice are prone to hyperoxic lung injury with respect to lung morphology, but severity of lung phenotype is partially attenuated by better preserved alveolar type II cell number and SPC production.
Abstract No. 329

**Alpha1-antitrypsin prevents oxidative activation of human neutrophils**

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Although alpha1-Antitrypsin (A1AT) is mostly viewed as an inhibitor of neutrophil elastase, A1AT is also an acute phase glycoprotein with broad anti-inflammatory properties. Among others, A1AT has been suggested as one other hemin scavenger protein and inhibitor of bacterial lipopolysaccharide (LPS) effects. Plasma hemoglobin released after rupture of red blood cells is associated with an adverse clinical prognosis in various pathologies. Hemoglobin is a source of free hemin, and high plasma levels of hemin (up to 20 µM) have been reported in patients with bacterial infections and ischemia-reperfusion injury. High levels of hemin cause oxidative stress and inflammation if not neutralized immediately.

We aimed to investigate if A1AT protects neutrophils from hemin and LPS-induced oxidative activation. A short-term exposure of freshly isolated human blood neutrophils to 4 µM hemin resulted in cell spreading, surface expression of vimentin, ROS, expression of heme oxygenase 1 (HO-1), release of interleukin 8 (IL-8), and enhanced neutrophil adhesion to human endothelial cells. Consequently, the phosphorylation of protein kinase C (PKC) occurred within 25 min. In parallel, neutrophil exposure to LPS significantly increased nitric oxide (NO) production and interleukin 1β (IL-1β) release and expression. Under the same experimental conditions, addition of 1 mg/ml A1AT markedly reduced or abolished neutrophil-activating effects of hemin and LPS. In a mouse model of acute kidney injury plus injection of hemin, monotherapy with 4 mg/mouse A1AT significantly lowered serum levels of free hemin at 2 h after surgery, and reduced infiltration of neutrophils. It is important to point out, that the oxidized form of A1AT, without anti-elastase activity, still neutralized neutrophil activating effects of hemin and LPS. It means that even if a large fraction of A1AT would lose anti-elastase activity, the protein still would be able to neutralize neutrophil-activating effects of hemin and LPS.
Abstract No. 330

Reduced Oxygenation Capacity of RDS Can be Achieved by Repetitive Saline Lavages Ex Vivo

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Respiratory Distress Syndrome (RDS) occurs in premature infants within the first few minutes of life when infants exhibit symptoms related to severe respiratory failure. Arterial blood gas analysis can show hypoxemia and hypercapnia that can cause respiratory acidosis and systemic metabolic acidosis. The primary cause for RDS is a diminished capacity in pulmonary surfactant production. The beneficial effects of surfactant treatment for the prevention or cure of RDS could be shown in several studies (RODRIGUEZ, 2003; SOLL and OZEK, 2009; WILLSON and NOTTER, 2011). Often, the rat lung lavage (RLL) model is used to test the effectiveness of new lung surfactant formulations for the treatment of RDS. In this secondary surfactant deficiency model, repetitive lung lavages are performed in anesthetized, tracheostomized and pressure-controlled ventilated animals. For the refinement and reduction of the RLL animal tests, the establishment of an ex vivo model such as the isolated perfused rat lung (IPL) is desirable.

We tested the effect of saline lavage procedures using IPLs. Lungs of rats were ventilated ex vivo with negative conditions (end-inspiratory pressure (P_{insp})/end-expiratory pressure of -12/-3 cmH$_2$O), an inspiration : expiration ratio of 1 : 1 and 100% oxygen at a respiratory rate of 80 breaths/min. Every 5 min one deep breath with a P_{insp} of 20 cmH$_2$O was performed to reduce atelectasis formation. Up to 6 lavages with 0,9% NaCl-solution caused a decreasing O$_2$ level of at least 200 mmHg.

For the treatment of RDS, market authorization of efficient exogenous surfactant preparations is crucial. Our data show that we are able to imitate a reduced oxygenation status in IPLs as it is seen in RDS. This new ex vivo test shall replace the currently used in vivo surfactant formulation testings and thus lead to a sensitive method to test the efficacy of RDS treatments.
Abstract No. 331

Global profiling of bronchial epithelial cell response to Streptococcus pneumoniae

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Streptococcus pneumoniae (S. pneumoniae) is a gram-positive bacterium that usually colonizes the human nasopharynx, but it is also an important pathogen causing fatal infections, such as pneumonia. The process of streptococci infections is influenced by both, host and pathogen factors. Since nowadays targeted bacterial factors possess only limited therapeutic potential we investigated this host-pathogen-relationship in more detail. Here, we aim to distinguish the human host response in gram-positive infections compared to specific S. pneumoniae infections in bronchial epithelial cells. Therefore, high-throughput profiling of microRNAs (miRNAs), mRNAs and proteins of a human bronchial epithelial cell line (BEAS-2B) infected with S. pneumoniae D39 wild-type (for 9 h or 16 h) compared to mock-infected or lipopeptide stimulated cells was performed.

The miRNA sequencing and annotation revealed 75 significantly regulated miRNAs. Several of these miRNAs were deregulated in a time and also in a treatment dependent manner including streptococcal specific candidates. Moreover, microarrays were performed to examine mRNA expression resulting in nearly 10,000 detected mRNAs in BEAS-2B cells. More than 2,000 or 5,000 of these mRNAs were significantly deregulated 9 h or 16 h post treatment, respectively. The amount of streptococcal specific regulated mRNAs increased over time from 22% up to 60% in this analysis. Besides that, the proteomic analysis was performed via stable isotope labeling with amino acids in cell culture (SILAC) method. In at least 2 of 3 biological replicates we detected and quantified more than 2,300 proteins comprising 38 significantly deregulated proteins, mainly 16 h post infection.

These data are now combined to create a profound bioinformatics model of the human host response specific to S. pneumoniae. This model will extent the knowledge of pneumococcal infections in human bronchial epithelial cells and may reveal potential new therapeutic approaches.
Disease Area Diffuse Parenchymal Lung Disease (DPLD): Abstract No. 401 - 457
Role of the COX2- PGE2 axis in infection-induced pulmonary fibrosis exacerbation in mice

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Idiopathic pulmonary fibrosis (IPF) is a severe chronic and progressive diffuse parenchymal lung disease associated with high morbidity and mortality. Acute exacerbation is a common complication in patients with IPF (AE-IPF). We recently showed that the lung pathogen S. pneumoniae through its virulence factor Pneumolysin (Ply) is able to trigger acute exacerbation in a model of AdTGFβ- induced pulmonary fibrosis in mice. Prostaglandin E2 (PGE2) is an antifibrotic eicosanoid released in fibrotic lungs in a cyclooxygenase-2 (COX2) dependent manner. Here we examined the role of the COX2-PGE2 axis in infection-induced fibrosis exacerbation in mice. Using cell sorting in conjunction with real-time PCR and ELISA techniques, we found that both type 2 alveolar epithelial cells as well as alveolar macrophages produced PGE2 during infection-induced pulmonary fibrosis exacerbation. Inhibition of COX2 by Parecoxib led to decreased alveolar PGE2 release and increased collagen deposition in infection-induced fibrosis exacerbation, which was accompanied by impaired clearance of S. pneumoniae in the lungs of mice.

Together, the data suggest that pharmacological blockade of the COX2-PGE2 axis impacts on pathogen elimination, thereby aggravating the fibrotic remodeling process.
Abstract No. 402

Transcriptomic profiles of IPF BAL cells treated with antifibrotic drugs

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Introduction: Idiopathic Pulmonary Fibrosis (IPF) is a chronic and progressive disease characterized by the destruction of the alveolar architecture and a consecutive decline of pulmonary function. Pirfenidone and Nintedanib are the only drugs approved for the treatment of IPF. The exact action mode of pirfenidone is not yet fully understood, but evidence suggests that pirfenidone blocks TGF-β, and TNF-α signaling. Nintedanib is a multi-tyrosine kinase inhibitor mainly targeting VEGFR, FGFR, and PDGFR signaling.

Methods: BAL cells of 10 patients with IPF were obtained during routine diagnostic work-up and cultured for 24h in the presence of either pirfenidone (1mM), nintedanib (1µM), TGF-β (2ng/ml), or the TGF-β inhibitor (SB431542, 10µM). In three patients BAL cells were harvested at initial diagnosis (baseline) and during treatment with pirfenidone. Cells were harvested in Qiazol and RNA isolated RNAeasy minikit (Qiagen). The transcriptome was analyzed by Agilent 2100 Bioanalyzer using SurePrint G3 Human Gene Expression v3 8x60K. In total 58339 genes were tested.

Results: While nintedanib treatment resulted in 5427 differentially expressed genes, we found 3745 changes in pirfenidone treatment comparing with unstimulated BAL cells. In vivo analysis revealed 2498 transcripts differently expressed between baseline and follow-up during pirfenidone treatment. Enrichment and clustering analysis pointed out common gene ontologies for the observed conditions, such as cancer, organismal injury and abnormalities, and immunological disorders. As top canonical pathways were cytotoxic T Lymphocyte-mediated apoptosis, trans-farnesyl diphosphate biosynthesis, role of JAK family kinases in IL-6-type cytokine signaling, regulation of eIF4 and p70S6K signaling, and protein ubiquitination pathway identified.

Conclusions: Based on this finding we are interested to establish changes in BAL cell gene expression as a read-out for early clinical trials testing new compounds.
SERUM METABOLOMICS OF THERAPEUTIC TREATMENTS OF IDIOPATHIC PULMONARY FIBROSIS

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Idiopathic Pulmonary Fibrosis (IPF) is a chronic and lethal interstitial lung disease characterized by deterioration of alveolar architecture and decline of respiratory function due to progressive parenchymal fibrosis. Recent data indicate changes in the metabolome of IPF lungs. Particularly the following pathways were shown to be altered in IPF: adenosine triphosphate degradation, glycolysis, mitochondrial beta-oxidation, tricarboxylic acid cycle, glutathione biosynthesis, sphingolipids, arginine, ornithine aminotransferase, and glutamate/aspartate metabolism.

In this study we investigated the serum metabolome of 115 patients with IPF and 16 healthy age-matched controls at initial diagnosis (baseline) and 4-10 weeks after start of therapy with either pirfenidone or nintedanib. We analyzed 188 different endogenous metabolites by the Biocrates AbsoluteIDQ p180 Kit and quantitative mass spectrometry (MS).

We found a significant correlation between the expression of several serum metabolites at baseline and clinical parameters. In the overall population we found correlations among GAP index and acylcarnitines and glycerophospholipids and between DLCO and acylcarnitines. In particular, C16 and histidine are the top hits (multiple R-squad value of 0.5, P=9.1E-04) in correlation with GAPindex. Treatment with pirfenidone modulated the expression of 73 metabolites (p<0.05, including amino acids, glycerophospholipids, and sphingolipids) while treatment with nintedanib modulated the expression of 40 metabolites (p<0.05, as acylcarnitines, biogenic amine, glycerophospholipids, and sphingolipids). Further analysis showed that early changes in metabolome correlated with long term outcome and incidence of disease progression.

Treatment with either pirfenidone or nintedanib lead to substantial changes in the serum metabolome which may be useful to predict treatment outcome.

Decellularized Precision Cut Lung Slices as a Model to Study in Vitro Lung Regeneration

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Idiopathic lung fibrosis is a disease characterized by continuous injury and consequent repair of the distal epithelial lung compartment, which is facilitated by cells with regenerative capacity from the conducting airways and the alveolar compartment. Our objective was to establish a model to assess the regenerative potential of various lung epithelial populations using decellularized mouse precision cut lung slices (PCLS). To achieve complete decellularization of mouse lung scaffolds, several detergent based protocols were compared in terms of efficiency of cell removal, structural integrity of the tissue and preservation of extracellular matrix proteins. To that end we compared the before mentioned parameters in protocols involving sodium dodecyl sulfate (SDS), Triton X 100, Tween 20, Sodium Deoxycholate (SDC) and CHAPS. Out of all, CHAPS decellularization achieved the best cell removal while minimizing structural damage and loss of structural proteins. We further tested the ability of CHAPS decellularized matrices to support the growth of various lung cell types by culturing total lung cells (mouse and human) for up to 21 days. Recellularization and proliferation of mesenchymal and epithelial cells was successfully achieved, demonstrating that decellularized PCLS provide a viable in vitro assay to study lung regeneration.
Abstract No. 405

**HMGA2 mediated histone deposition is required for TGFB1 induced transcription.**

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**Objectives:** During transcriptional activation, chromatin undergoes structural changes with the help of various proteins such as chromatin remodeling factors and histone modifiers in order to make the DNA accessible for the transcription factors and RNA polymerase. The replacement of canonical histones by histone variants and deposition of histones are chromatin regulating mechanisms devised by cells to dynamically regulate gene expression. New evidence suggest that TGFB1 induced transcription requires the phosphorylation of H2A.X at serine 139. Although we have shown that HMGA2 was required for TGFB1 induced transcription, the mechanism underlying our results was still not completely elucidated.

**Results:** We combined ChIP- and MNase-sequencing analyzing the *Hmga2* knock-out mice and found that HMGA2 is required for promoter specific deposition of the histone variant H2A.X. We further demonstrated that the intrinsic lyase activity of HMGA2 is required for this process. Consequently, the promoter specific H2A.X deposition resulted in an accumulation of active RNA Polymerase II at these promoters. Stimulation with TGFB1 resulted in increased expression of those “primed” target genes, whereas cells expressing no *Hmga2* where not able to respond.

**Conclusions:** We conclude that HMGA2 “primes” specific genes via histone deposition at promoters that are activated upon TGFB1 stimulation.
Abstract No. 406

Pharmacometabolic response to pirfenidone treatment in pulmonary fibrosis detected by high resolution MALDI-FTICR-mass spectrometry imaging

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Idiopathic pulmonary fibrosis is a fatal condition with limited life expectancy and response to available therapies. Pirfenidone has been approved for the treatment of IPF, but we know little about distinct metabolic changes in the lung upon pirfenidone treatment.

In this study, we used high resolution MALDI-FTICR-mass spectrometry imaging (MSI) to simultaneously detect, visualize, and quantify in situ endogenous and exogenous metabolites in lungs of mice and humans subjected to experimental fibrosis or with IPF, respectively, and assessed the effect of pirfenidone treatment on these levels.

Metabolic pathway analysis and endogenous metabolite quantification revealed that pirfenidone treatment restored redox imbalance and glycolysis in IPF tissue, and downregulates ascorbate and aldarate metabolism, thereby likely contributing to in situ modulation of collagen processing. As such, we detected specific alterations of metabolite pathways in fibrosis, and most importantly, metabolic recalibration following pirfenidone treatment. Our study demonstrates, for the first time, in situ metabolic alterations during fibrosis and analyzes the pharmacometabolic effect of pirfenidone in fibrotic tissue of mice and humans. In detail, we demonstrated an increase of pirfenidone and its related metabolites in fibrotic tissue using high-resolution in situ mass spectrometry imaging. We further detected the mass spectra of fibrotic areas, to unequivocally assess metabolite detection in specific areas of interest. Next, using this technology, we are able to completely dissect metabolic pathways simultaneously in multiple samples and determine spatial distribution, as well as quantifying the intensity of detection. Last, we identified overlapping and exclusive metabolic fingerprints that characterize the fibrotic response in human and mice, together with the pharmacometabolic response to pirfenidone in fibrosis.

Together, these results highlight the suitability of high resolution MALDI-FTICR-MSI to decipher therapeutic effects of pirfenidone and will help clarify disease mechanisms of pulmonary fibrosis that may contribute to improvement of currently available therapies for IPF.
Abstract No. 407

Development of a cell-based assay to identify small molecule correctors for rescue of mutant ABCA3 by High-Throughput Screening

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Introduction: Mutations in the ATP-binding cassette (ABC), subfamily A, member 3 (ABCA3) gene are the most common genetic cause of respiratory distress syndrome in newborns and interstitial lung disease in children. Currently these is no cure available. ABCA3 is a lipid transporter expressed in alveolar type II cells and localized in the limiting membrane of lamellar bodies. It is crucial for pulmonary surfactant storage and homeostasis. Recently we identified small molecules (C13, C17) which rescued different mutant ABCA3 proteins in vitro.

Methods: A549 cells stably expressing HA-tagged wildtype ABCA3 or variant K1388N were treated with the correctors C13 and C17 in 384-well CellCarrier plates. Cells were analyzed by immunofluorescence staining. Plate and liquid handling was performed using a HTS platform system composed of a Sciclone G3 Liquid Handler with a Mitsubishi robotic arm, a MultiFlo Dispenser and a Cytomat Incubator. Image acquisition and image-based quantification were performed using an Operetta/Columbus high-content imaging platform. To identify novel small molecules for correction indicated by restoration of processing and intracellular localization a collection of food and drug administration (FDA)-approved small molecule compounds will be tested.

Results: The functional correction of ABCA3 variant K1388N by C13 and C17 was stably reproduced in an optimized automated cell-based HTS-Assay. In a next step, FDA-approved compound libraries will be tested and primary hits will be validated in primary assays and by determination of toxicity.

Conclusion: The development of a cell-based HTS-assay to identify small molecule correctors for rescue of mutant ABCA3 by screening FDA-approved small molecule libraries is an important step towards the development of targeted therapy for the treatment of children suffering from respiratory distress syndrome or interstitial lung disease.

Supported by grants from the DZL and Helmholtz Zentrum München.
Abstract No. 408

A phenotypic drug discovery pipeline for novel antifibrotic IPF therapies

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Idiopathic lung fibrosis (IPF) is a progressive interstitial lung disease with a median patient survival of 3-5 years. No approved pharmacotherapeutic is currently able to stop disease progression in IPF patients. Therefore, discovery of novel therapeutic targets and active pharmaceutical compounds for IPF treatment represents a major medical need. We developed a high-throughput ECM deposition assay based on 96- and 384 well-plates, which enables the 3D quantification of the insolubly deposited extracellular matrix (ECM) components. In the assay, IPF tissue derived fibroblasts were activated by TGFβ1 to trigger transdifferentiation into myofibroblasts and a concomitant increase in ECM deposition, both defining hallmarks of IPF. Live immunolabeling and automated confocal imaging together with automated image analysis confidently measured the increase of ECM deposition after activation. As proof of concept, we successfully quantified the inhibition of collagen I deposition by antagonizing collagen-specific proline-4-hydroxylase, a possible therapeutic target in IPF, with Ethyl-3,4-dihydroxy-benzoate (EDHB). The optimized ECM deposition assay will enable the high-throughput screening of potential antifibrotic therapeutics in large chemical libraries. To validate possible hits from the screen, we utilized an ex-vivo tissue culture system1 of human precision-cut-lung slices (PCLS) that recapitulate distinct features of lung fibrosis, such as ECM remodeling. Within the PCLS we measure abundancy of ECM molecules, such as collagen I, by 3D confocal immunofluorescence microscopy. We use this model to assess the efficacy of potential antifibotics, discovered by the ECM deposition assay, for inhibition of ECM remodeling. In conclusion, we established a phenotypic screening pipeline for the discovery of novel potential antifibrotic pharmacotherapeutics. Drug candidates identified in the ECM deposition assay are validated in an ex-vivo fibrosis model prior to selecting the best candidate for in-vivo validation. This system will offer an innovative approach for unbiased antifibrotic drug discovery and validation.

1 Alsafadi et al. (2017), AJP Lung
Abstract No. 409

Fractalkine/CX3CR1 axis drives kinetic changes of monocytes in fibrotic Interstitial Lung Disease patients

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Rationale: Interstitial lung disease is a group of fibrotic disorders with progressive extracellular matrix deposition, occasionally associated to inflammation. Monocyte subtypes correlate with the canonical expression of CCR2 and CX3CR1. To date, the role of non-classical monocytes and fractalkine/CX3CR1 in ILD is unclear.

Methods: Flow cytometry analysis was performed to analyze circulating monocytes. CCL2 and fractalkine were measured via ELISA in plasma and tissue. Monocytes were detected in tissue by immunofluorescence. For functional assays, monocytes were bead-sorted and cultured alone, or with endothelial cells.

Results: Non-classical monocytes in IPF and non-IPF ILD were decreased compared with control (p<0.05, and p<0.01, respectively). Only in non-IPF ILD decreases of non-classical monocytes correlated with DLCO decline (p=0.0036, r=0.3369). Non-classical monocytes showed decreased CX3CR1, and increased scavenger receptor CD163 in non-IPF ILD compared with control (p<0.01). Circulating amounts of CCL2 were increased in non-IPF ILD (p<0.001), while plasma levels of CX3CL1 did not differ from control. Non-classical monocytes were increased in the interstitium of non-IPF ILD (p<0.05), not in control. To investigate the driver of non-classical monocyte migration into fibrotic lungs, we quantified CCL2 and fractalkine in lung homogenates. We observed increased levels of fractalkine in non-IPF ILD (p<0.001), but not CCL2. To understand the chemoattractant potential and receptor responsiveness, we performed functional assays in presence or absence of both ligands. In co-culture with endothelial cells neither CCL2 nor fractalkine showed influence in non-classical monocyte adhesion. However, in non-IPF ILD patients only, non-classical monocyte migration was significantly increased in the presence of fractalkine (p<0.01), but not in the presence of CCL2.

Conclusions: This study suggests that increased of non-classical monocyte functional responsiveness to fractalkine, mediates their enhanced migration into the lung parenchyma of non-IPF ILD patients.
Abstract No. 410

Quantitative Proteomics Reveals Novel Fibrotic Networks of Myeloid-Derived Suppressor Cell and Monocytes in IPF

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Rationale: Idiopathic pulmonary fibrosis (IPF) is a fibroproliferative lung disease with irreversible loss of lung function. Myeloid-derived suppressor cells (MDSC) are pathologically activated immature myeloid cells, which suppress immune responses in cancer, autoimmunity, and other inflammatory conditions.

Methods: Flow cytometry analysis was performed to analyze MDSC in peripheral blood. Label-free quantitative MS-analysis was used in sorted monocytes and MDSC from human blood. Receptor-ligand interactions datasets were used to identify paracrine and autocrine signals.

Results: MDSC are increased, functionally active, and reflect disease status in IPF. Monocytic MDSC are the predominant subtype in IPF. We identified and quantified more than 7000 proteins. Principal component analysis unequivocally discriminated both cell types, showing that proteome differences between them are larger than the biological variations between the donors. 4345 proteins were detected in at least 2/3 of all samples per cell type, and subject of further analysis. Comparing the sets of proteins identified in the two cell types we found 502 MDSC enriched and 1224 monocyte enriched proteins (2 to >30 log10-transformed LFQ intensity ratios). In the combined dataset 200 ligands and 153 receptors were detected. From the cell-to-cell communication analysis we identified both autocrine signaling edges from monocyte to monocyte (339), MDSC to MDSC (290), and paracrine signaling edges from monocyte to MDSC (311) and MDSC to monocyte (316). Specific ligands predicted to signal from monocyte to MDSC included: ANXA1, CCL18, CXCL2, HSP90AA1, ICAM1, TGFB2, amongst others. Ligands from MDSC to monocyte included: COL1A1, FN1, HLA-C, HSPG2, MMP1, S100A8-9, TGFB1, amongst others. Finally, FACS staining confirmed the surface expression of the cognate expressed receptors in both populations.

Conclusions: This study network analysis where autocrine and paracrine signals from and between monocytes and MDSC might lead to identification of novel proteins useful for therapeutic targeting of MDSC and monocytes in IPF.
Abstract No. 411

**Autophagy in lung fibrosis: Exploring the mitophagy pathways**

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Autophagy is a lysosomal quality control mechanism that aims at cell survival, but severe autophagic stress may lead to apoptosis. Defective autophagy is implicated in the pathology of idiopathic pulmonary fibrosis (IPF) and in other forms of lung fibrosis. Here, we aim to dissect the role of mitochondrial autophagy (mitophagy) proteins in IPF and in the amiodarone (AD) model of lung fibrosis. Differential expression of the mitophagy proteins PINK1, Parkin and BCL2L13 was observed in IPF lungs and in AD-treated mouse lung epithelial cells (MLE12). In MLE12 cells treated with AD, BCL2L13 that was localized to mitochondria also colocalized with autophagosomal marker LC3B and lysosomal marker Lamp1, indicating the activation of BCL2L13-dependent mitophagy. Similarly, the selective autophagy adaptor protein, sequestosome 1 (p62) was significantly increased in IPF lungs and in response to AD treatment. Of note, in AD-treated MLE12 cells, mitophagy was also activated via binding of p62 to dysfunctional mitochondria in addition to the increased interaction of p62 with Keap1 and localization of Keap1 to p62 positive vacuolar structures. However, Nrf2, a competitive binding partner of Keap1 and a major transcriptional factor regulating antioxidant proteins, was significantly reduced in epithelial cells following AD treatment. Hence stimulation of Nrf2 may regulate the p62-Keap1 binding and subsequent mitophagy pathways in AD-treated cells. Our study suggests that targeting the mitochondrial-lysosomal axis may have therapeutic importance in the context of lung fibrosis.
Abstract No. 412

**Analysis of disease-associated uncharacterized SP-A2 variants occurring in idiopathic pulmonary fibrosis (IPF) concerning general surfactant properties and their influence on ER-stress**

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Surfactant protein A (SP-A) is a major protein of pulmonary surfactant, expressed in type-II alveolar epithelial cells (AECII) and Clara cells. It plays a key role in maintaining the integrity of the air spaces in the lung, thereby stabilizing the gas-exchanging surface of the alveoli. Recently, Garcia CK et al. showed that IPF-associated SFTPA2 variants induce the expression of adaptive ER-stress markers. We aim to elucidate whether newly identified uncharacterized SFTPA2 mutations behave different compared to wild-type SP-A2 concerning expression, secretion and post-translational modification, and whether they cause induction of severe ER-stress and subsequent apoptosis in AECII-like cells and Clara cells. For this issue, a detailed description of several SFTPA2 variants is indispensable. All point mutations were successfully introduced within SP-A2 cDNA and expressed to a comparable extent in A549 and H441 cells. Importantly, the pro-apoptotic ER-stress transcription factor CHOP was significantly induced on mRNA level in response to overexpression of the different SFTPA2 mutants. The expression of SFTPA2 mutations V178M and G231R caused an increase in CHOP expression up to 8-fold as compared to wild-typ SP-A2, 24h after transfection. Pro-apoptotic downstream targets of CHOP showed slight induction upon SFTPA2 variant overexpression, whereas upstream ER-stress transducers were not altered. In addition, preliminary studies indicate impaired secretion of SFTPA2 variants in A549 cells.

Our results indicate a link between SFTPA2 mutations and pro-apoptotic ER-stress markers in IPF. The pathways leading to CHOP induction have to be figured out.
Abstract No. 413

Specific induction of pro-fibrotic biomarker in human ex vivo lung tissue slices

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Pulmonary fibrosis covers a scope of rapidly progressing lung disease, characterized by uncontrolled deposition of extracellular matrix (ECM), excessive proliferation of fibroblasts and destruction of cellular architecture of the lung. To date, the development of new therapies is hampered by the lack of animal models that do not entirely reflect all features of the disease as found in the patients. With the use of PCLS, the aim was to identify specific cell signaling pathways and pro-fibrotic mediators involved during pulmonary fibrosis in order to test the efficacy of pharmacological approaches. PCLS were prepared from tumor-free lung tissue from cancer patients and were cultured in the presence of TGF-β, TNF-α or combination of both to induce a pro-fibrotic profile. Analysis of the mRNA profile revealed up-regulation of important pro-fibrotic genes in PCLS stimulated with TGF-β, and TNF-α. Importantly, this up-regulation was generally concentration dependent. In addition, single stimulation with TNF-α alone lead to the up-regulation of IL-1β mRNA levels, upon others, whereas TGF-β single stimulation showed no effect on mRNA levels of this cytokine. Stimulation of PCLS with either TNF-α or TGF-β showed up-regulation of PAI-1. Importantly, the combination of both lead to a further amplification of mRNA levels. In general, stimulation of human PCLS with TNF-α and TGF-β leads to up-regulation of specific pattern of pro-fibrotic genes as compared to medium controls. Several integrins, e.g. ITGAV or ITGB6 were up to 5-fold upregulated whereas others, e.g. ITGA3 were downregulated. Ex vivo treatment with Pirfenidone could clearly revert the TGF-β and TNF-α-mediated up- or downregulation. Overall, we describe here the induction of a specific pro-fibrotic biomarker pattern in PCLS from TGF β and TNF-α stimulated human lung tissue with high translational relevance. Modulation of the pro-fibrotic pattern by ex vivo treatment with anti-fibrotic medications was efficient.
Abstract No. 414

CXCR4 Inhibition by the ibody AD114 blocks bronchosphere formation in a 3D organoid model.

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Background: Idiopathic pulmonary fibrosis is a fatal disease with mean survival time of 3 years. Our previous work suggested an important role of airway basal cells (ABC) in IPF.

Objectives: In vitro testing for effects of the i-body AD-114 [AdAlta/Australia], CXCL12, which is the natural ligand of CXCR4, pirfenidone and nintedanib in a 3D organoid model based on ABCs derived from IPF patients.

Methods: ABCs were harvested during bronchoscopy via brushing of the bronchi of the right lower lobe. Purity of IPF- ABC isolation procedure was checked by immunocytoology. IPF ABCs were cultivated with or without the presence of lung fibroblasts in a transwell system. Cells were treated with either the ibody AD-114 (AdAlta/Australia) [5µM], CXCL12 (SDF-1) [50ng/ml], pirfenidone [2mM] or nintedanib [1µM]. Sphere formation was counted by bright field microscopy once a week. Cell proliferation was quantified by MTT assay on day 21 and cell viability checked by confocal laser microscopy.

Results: Unstimulated IPF- ABCs generated bronchospheres which was highly up-regulated in the presence of fibroblasts. CXCL12 stimulation resulted in larger spheres but sphere counts were unchanged. Blockade of the CXCR4 pathway by the i-body AD-114 lead to a dose-dependent and complete inhibition of bronchosphere formation [p<0.05]. Both pirfenidone and nintedanib significantly reduced sphere counts but only slightly. Similar results were obtained in the co-culture system with fibroblasts.

Conclusion: CXCR4 inhibition by the ibody AD-114 abolishes bronchosphere formation of IPF- ABCs with or without the presence of IPF fibroblasts. CXCR4 may be a new target for IPF treatment.
Abstract No. 415

Highly significant differential expression of 9 mRNAs & 13 miRNAs in a random forest analysis of Endobronchial Epithelial Lining Fluid and Bronchioalveolar Lavage in patients with IPF

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We assessed endobronchial epithelial lining fluid (EELF) sampled by the minimally invasive endoscopic method of bronchoscopic microsampling (BMS) for novel molecular biomarkers in idiopathic pulmonary fibrosis.

EELF was collected from 14 patients by BMS from subsegmental bronchi (generation 5-7). BAL from the same patients was sampled from the middle lobe or lingula. Total RNA extraction and quality assessment was performed as previously described. Transcriptome expression analysis was performed with GeneChip® miRNA 3.0 and with Human Transcriptome Array (HTA) 2.0 (both Affymetrix). Random forest (RF) analysis was used to define a set of differentially expressed microRNAs and mRNA between BAL and EELF. To find the relation between these differentially expressed miRNA and mRNA genes, canonical correlation analysis (CCA) was performed.

A total of 40 samples were analyzed for microRNA and mRNA expression. Initial RF analysis revealed a total number of respectively 595 and 207 differentially expressed mRNA and microRNA genes between EELF and BAL. The final RF analysis led to a set of respectively 9 and 13 most differentially expressed and statistically significant (p-value <0.001) mRNA and microRNA genes between EELF and BAL. These two sets could differentiate EELF and BAL on microRNA and mRNA level using principal component analysis. CCA analysis showed a significant correlation (R=0.976 with p-value<0.0001) between the two differentiating sets of genes.

A significant differential mRNA and miRNA expression was detected between BAL and EELF samples from IPF patients. The observation that the two differentiating sets of mRNA and microRNA genes were significantly correlated indicates the involvement of similar pathways. Therefore, our results suggest that EELF could be an additional biomaterial worth screening for potential biomarkers in IPF.
Abstract No. 416

Epigenetic modulation of pro-coagulant and fibrinolytic gene expression in fibroblasts from patients with idiopathic pulmonary fibrosis (IPF) by histone deacetylase (HDAC)-inhibitors

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Uncontrolled pro-coagulant signalling has been shown to contribute to excessive inflammatory and fibroproliferative responses in IPF. Moreover, epithelial cells and fibroblasts have been shown to represent a prominent source of extravascular pro-coagulant activity due to abnormally enhanced expression of coagulants. The aim of the study was to test whether pharmacological intervention through 15h/30h-treatment with HDAC-inhibitors panobinostat (100 nM) or valproic acid (VPA, 2 mM) can modulate the pro-coagulant activity in IPF-lungs. Both, panobinostat and VPA significantly increased t-PA (PLAT) mRNA and protein expression in treated (primary) IPF-fibroblasts (n=6), indicating a change in a profibrinolytic direction. This was in line with significantly decreased mRNA- and protein levels of PAI-1, the main inhibitor of fibrinolysis. In addition, expression of proteinase-activated receptor-1 (PAR1), the major signalling receptor for thrombin, and which is reported to be highly overexpressed in fibroblastic foci of IPF lungs, was significantly reduced in response to both treatments. Interestingly, only 15h-VPA-treated IPF-fibroblasts indicated increased expression of u-PA (PLAU) and u-PAR (PLAUR), as compared to vehicle, whereas panobinostat suppressed the expression of both fibrinolytic enzymes. Moreover, protein level for the plasmin-fragment angiostatin could be significantly enhanced in response to VPA-treatment.

Interestingly, both HDAC-inhibitors also up-regulated significantly the expression of matrix metalloproteinase 9 (MMP9) and of all four tissue inhibitors of metalloproteinases (TIMP1-4), but only TIMP-3, a tumor suppressor, has a prominent anti-fibrotic function. We conclude, that excessive pro-coagulant activity in IPF may be epigenetically modulated towards a profibrinolytic direction, with use of HDAC-inhibitors, but needs further investigations. This includes the HDAC-inhibitor-treatment of fibrotic alveolar epithelial cells.
Abstract No. 417

Susceptibility of LC3B knockout mice to lung injury and fibrosis

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Idiopathic pulmonary fibrosis (IPF) is a disease with a remarkable age-related onset which may be triggered by chronic lung alveolar epithelial cell type II (AECII) injury and apoptosis. AECII are classical secretory cells which contain lamellar bodies that are lysosome related organelles and are primarily responsible to store and secrete lung surfactant. Recently, the principle role of autophagy, a lysosome dependent protein quality control mechanism has been studied in the development of lung fibrosis, both in humans as well as in animal models. Yeast Atg8 or mammalian microtubule-associated protein 1 light chain 3 beta (MAP1LC3B/LC3B) is an important autophagy related protein and its lipidated form, LC3BII is a reliable marker of the autophagosomes. In this study, we aim to decipher the involvement of this protein in the development of lung fibrosis. A systematic analysis of the LC3B-/- mice lungs revealed that aged LC3B-/- mice showed increased cellularity, smaller lamellar body profiles, increased apoptosis of AECII paralleled with surfactant alterations, increased lysosomal and endoplasmic reticulum stress providing clues on the importance of this distal autophagy protein in lung fibrosis development. Further, in vitro knockdown of LC3B sensitized mouse lung epithelial cells to bleomycin induced apoptosis. In vivo, LC3B-/- mice displayed increased susceptibility to bleomycin induced lung injury and fibrosis. We conclude that LC3B plays essential roles in AECII and protects the alveolar epithelial cells from bleomycin induced lung injury and fibrosis.
Abstract No. 418

Anti-diabetic drug metformin imposes anti-fibrotic effects by controlling fibroblast plasticity

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Objective: We have recently demonstrated an interconversion between lipogenic and myogenic fibroblastic phenotypes during fibrosis formation and resolution in the lung. The current study aims at investigating potential therapeutic effects of anti-diabetic drug metformin in idiopathic pulmonary fibrosis (IPF).

Results: Metformin treatment induces lipogenic differentiation in primary human lung fibroblasts as evident from marker gene expression (PLIN2, PPARg and SREBF1) and neutral lipid staining. Similar results are observed in precision-cut lung slices (PCLS) prepared from human lung tissues, where metformin also significantly reduces collagen expression. Furthermore, metformin-treated PCLS display general improvement in alveolar structure. Interestingly, metformin also counteracts TGFβ1-induced myogenic differentiation and favors lipogenic differentiation. Gain and loss-of-function experiments in vitro show that the effect of metformin is partially mediated by AMPK signaling pathway.

Conclusion: Metformin treatment reveals robust anti-fibrotic effects in vitro. Future studies will employ the bleomycin model of lung fibrosis in mice to investigate this therapeutic potential in the context of lineage tracing during fibrosis formation and resolution.
Abstract No. 419

**Functional rescue of mutant ABCA3 by CFTR potentiators**

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ABCA3 is a phospholipid transporter expressed in alveolar type II pneumocytes and localized at the limiting membrane of lamellar bodies (LB). It is essential for the assembly of pulmonary surfactant and LB biogenesis. Mutations in the ABCA3 gene display a common genetic cause for respiratory distress syndrome in newborns and interstitial lung disease in children and adults.

The aim of this study is to test CFTR potentiators *in vitro* for different clinically relevant ABCA3 mutations that lead to functional impairment.

A549 cells transfected with HA-tagged wildtype or mutated ABCA3, were treated with a range of different potentiators to identify molecules that are able to restore ABCA3 function assessed by the transport of TopFluor-conjugated phosphatidylcholine (TopF-PC) into ABCA3-positive vesicles resembling LBs.

We identified several potentiators that increased TopF-PC transport into ABCA3-positive vesicles and restored phospholipid transport function of different ABCA3 mutations.

The identification of molecules with the ability to restore function of mutated ABCA3 are an important step towards the development of drugs for the treatment of children suffering from respiratory distress syndrome or interstitial lung disease.

Supported by Deutsches Zentrum für Lungenforschung (DZL)
Abstract No. 420

**Surfactant dysfunction, alveolar collapse and airspace heterogeneity are linked with fibrotic septal wall remodeling in the TGF-b1 mouse model**

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Recent imaging studies in Idiopathic Pulmonary Fibrosis (IPF) showed that collapse of distal airspaces occurs in not (yet) remodeled areas of the lung. Mice lungs overexpressing a key mediator of pulmonary fibrosis, the transforming growth factor-b1 (TGF-b1), recapitulate this abnormality: surfactant dysfunction results in alveolar collapse preceding fibrotic remodeling and ultrastructural alterations of alveolar epithelial type II (AE2) cells including loss of the surface area of the apical membrane. Here, the hypothesis whether surfactant dysfunction related alveolar collapse due to TGF-b1 overexpression is linked with septal wall remodeling and AE2 cell abnormalities was investigated. Three and 6 days after gene-transfer of TGF-b1, mice received either intratracheal surfactant (Surf-groups: Curosurf®, 100mg/kg bodyweight) or 0.9% NaCl (Saline-groups). At days 7 (D7) and 14 (D14) lung mechanics was assessed followed by either design-based stereology at light and electron microscopy level or measurement of hydroxyproline level per lung. Compared to Saline, Surf showed significantly reduced tissue elastance while the number of open alveoli and the alveolar surface area was increased at D7. At D14, tissue elastance and alveolar number remained significantly improved. In addition, alveolar size variability and surface area of alveolar epithelial basal lamina was decreased in the Surf-group. While the volume of interstitial cells in septal walls did not differ, there was a significant decrease in the total volume of collagen fibrils in septal walls in the Surf-group. An inverse correlation could be established between open alveoli and the total volume of collagen fibrils in septal walls at D14. Furthermore, AE2 cells demonstrated a significantly increased surface area of apical membrane in Surf compared to Saline at D14. In conclusion, surfactant replacement modulates extracellular matrix remodeling of septal walls which correlates with reduced alveolar collapse. These antifibrotic effects might be due to a reduction of mechanical strain.
Abstract No. 421

Fibroblast migration is regulated by FKBP10 via synthesis of collagen VI

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Rationale: In idiopathic pulmonary fibrosis (IPF), fibroblasts gain a migratory phenotype excessively synthesize and deposit extracellular matrix (ECM), ultimately leading to alveolar scarring and loss of lung function. An important regulator of collagen synthesis and secretion in IPF is FK506-binding protein 10 (FKBP10). In this study we analyzed the effects of FKBP10 deficiency on primary human lung fibroblasts (phLF) on adhesion and migration.

Methods: Using siRNA technology, FKBP10 expression was downregulated in phLF in absence or presence of 2ng/ml transforming growth factor-β1 and 0.1mM 2-phosphoascorbate. Effects on adhesive and migratory properties of phLF were analyzed by an immunofluorescence (IF)-based attachment assay, a conventional scratch assay, and single cell tracking. Effects of FKBP10 deficiency on important key players in adhesion dynamics and on ECM proteins was examined by qPCR and Western Blot. IF microscopy in z-stack images and proximity ligation assays were used to analyze colocalisation. Results: Loss of FKBP10 significantly reduced adhesion and migration of phLF. Effects on migration were dependent on 2-phosphoascorbate, indicating collagen synthesis as the underlying mechanism. In response to FKBP10 knockdown, collagen VI expression was downregulated, contrary to the expression of key proteins of the focal adhesion complex, including talin-1, calpain-4, integrin-β1, coronin-1C and fibulin-1 which were unchanged or even upregulated. FKBP10 and collagen VI colocalized in phLF. Finally, coating of culture dishes with collagen VI, and to a lesser extent with collagen I, abolished inhibition of migration by FKBP10 deficiency. Conclusion: Deficiency of FKBP10 may lead to decreased migration by reduced biosynthesis of collagen VI.
Abstract No. 422

**A proteomic analysis of extracellular matrix from idiopathic pulmonary fibrosis patient-derived fibroblasts in response to transforming growth factor β1**

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**Rationale:** Idiopathic pulmonary fibrosis (IPF) is an irreversible interstitial lung disease characterized by excessive deposition of extracellular matrix (ECM) in the interstitium leading to progressive dyspnea. Transforming growth factor β1 (TGF-β1), a central profibrotic cytokine induces expression of many ECM components, but these effects have, to our knowledge, not been studied in a proteomic approach.

**Methods:** Primary human IPF fibroblasts were cultured with and without 2ng/ml TGF-β1 for 48h in presence of 0.1mM 2-phospho-L-ascorbic acid. For enrichment of ECM components, the insoluble material after protein extraction was pelleted and solubilized using 6M guanidinium chloride-containing buffer. ECM components were digested with LysC/trypsin and the resulting peptides analyzed by liquid-chromatography-tandem mass spectrometry in a quadrupole orbitrap mass spectrometer. Identification and label-free quantification was performed using Progenesis IQ and Mascot. Regulation of gene expression of selected targets was validated by qPCR and Western Blot analysis.

**Results:** We consistently detected 115 matrisomal proteins synthesized by IPF fibroblasts, including 58 core matrisome proteins and 57 matrisome-associated proteins, which is superior to many other similar in vitro studies. TGF-β1 significantly induced levels of 34 ECM proteins including known targets like fibronectin 1 and collagen I, but also previously unrecognized targets like SERPINE2 and SEMA7A. At the same time, TGF-β1 significantly reduced levels of 29 ECM proteins including the collagen VI chains, and laminins α4 and β2. As collagen chain stoichiometry has been shown to strongly influence cleavage efficiency by collagenases, in particular for collagen I, we also assessed abundance ratios for collagen chains, but found no significant changes. Interestingly, collagen chain stoichiometries argue for a number of previously unrecognized heterotrimeric and heterotypic collagen forms.

**Conclusions:** We provide the first comprehensive proteomic analysis of TGF-β1-induced changes in the ECM. TGF-β1 induced, but, notably, also downregulated expression of a multitude of ECM proteins and regulators in IPF fibroblasts.
Abstract No. 423

Comparison of the antifibrotic efficacy of the pan-histone deacetylase-inhibitor panobinostat versus the IPF-drug pirfenidone in fibroblasts from patients with idiopathic pulmonary fibrosis (IPF)

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Idiopathic pulmonary fibrosis (IPF) is an incurable lung disease with a poor prognosis. Pirfenidone is the first antifibrotic agent to be approved for IPF-treatment as it is able to slow down disease progression. Because epigenetic alterations are associated with IPF, histone deacetylase (HDAC)-inhibitors have recently been proven to attenuate fibrotic remodeling in vitro and in vivo. This study compared the effects of pirfenidone with the pan-HDAC-inhibitor panobinostat/LBH589, a FDA-approved drug for the treatment of multiple myeloma, head-to-head on survival, fibrotic activity and proliferation of primary IPF-fibroblasts in vitro.

24h-treatment of IPF-fibroblasts with pirfenidone (2.7 mM) or panobinostat (85 nmol) resulted in significantly downregulated expression of various extracellular matrix (ECM)-associated genes, in comparison to vehicle-treated cells. In addition, both drugs decreased protein level of phosphorylated (p)-STAT3, a transcription factor mediating profibrotic responses, in treated IPF-fibroblasts. Of note, an increase in histone acetylation was observed in response to both treatments, but was much more pronounced and excessive in panobinostat-treated IPF-fibroblasts. Panobinostat, but not pirfenidone, led to a significant suppression of proliferation in IPF-fibroblasts, as indicated by WST1-assay and markedly diminished level of cyclin-D1 and p-histone H3. Further, panobinostat-treatment decreased markedly the expression of survival-related genes Bcl-XL and BIRC5/survivin, and was associated with induction of ER-stress and apoptosis in IPF-fibroblasts. Importantly, pirfenidone-therapy led also to significant downregulation of the cancer-associated gene BIRC5, but was not associated with induction of pro-apoptotic ER-stress. In line with slightly increased chromatin-acetylation, pirfenidone reduced the expression of HDAC1 and -2.

We conclude that, beside other antifibrotic mechanisms, pirfenidone reduces profibrotic signaling also through weak epigenetic alterations in IPF-fibroblasts, thereby permitting survival of (altered) fibroblasts. In contrast, the anti-cancer drug panobinostat reduces profibrotic expression while inducing cell death in IPF-fibroblasts. We believe that HDAC-inhibitors such as panobinostat can present a novel therapeutic strategy (in addition to pirfenidone) for IPF.
Abstract No. 424

1. **Diagnosing diffuse parenchymal lung disease (DPLD) by non-invasive breath screening of exhaled volatile compounds using an electronic nose: a pilot study.**

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**Background:** The diffuse parenchymal lung diseases (DPLD) is a big group of pulmonary inflammatory and/or fibrosing diseases with diverse natural courses; some of them, i.e. idiopathic pulmonary fibrosis (IPF) are associated with high mortality rate. The patient’s survival depends on timing of correct diagnosis. We conducted this single-center trial to evaluate, if a rapid, point-of-care, and easy-to-use diagnostic tool, such as the electronic nose Aeonose®, would be able to differentiate between DPLDs and healthy state.

**Methods:** The electronic nose signatures of exhaled volatile compounds of 116 patients with DPLD were captured using the Aeonose® and compared to a cohort of 42 healthy subjects. Area under Curve (AUC) and Matthews correlation coefficient (MC) were used to interpret the Aeonose® data.

**Results:** The Aeonose® was able to differentiate between IPF-patients (n=62) and healthy controls, showing an AUC of 0.92 and MC of 0.71. In comparison between cryptogenic organizing pneumonitis (COP, n=30) vs. healthy, an AUC of 0.86 and MC of 0.7 were obtained. In case of patients with connective tissue diseases - ILD (CT-ILD, n=24) vs. healthy, an AUC of 0.89 and MC of 0.7 were encountered.

**Conclusions:** Based on exhaled volatile compounds, the Aeonose® shows promising potential in capturing disease-specific signatures in IPF, COP or CT-ILD and may therefore be suited to improve the efficacy and accuracy of a specific diagnosis in ILDs. However, additional studies validating these results in a larger cohort and more complex, multivariate statistical analyses are necessary to further document the usefulness of this device.
Abstract No. 425

**Diagnosis and management of patients with interstitial lung disease (ILD) in clinical practice in Germany: The EXCITING-ILD registry within the DZL**

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**Background & Methods:** The prospective ILD registry EXCITING collects data from all health care forms in Germany assessing characteristics, diagnosis, management and outcomes.

**Results:** Until 1/2017, 601 patients were included (61% male, mean 64 years, 54% current/ex-smokers). Median FVC was 76%, DLCO 54% and ILD severity as measured by GAP-ILD index was 0-1 29%, 2-3 27%, 4-5 26%, 6-8 18%. Diagnostic procedures enclosed 93% CT (46% HRCT), 70% BAL, 13% surgical lung biopsies, 38% cryo-TBBs. 59% were discussed multidisciplinary. The following ILDs subtypes were diagnosed: IIP 40% (IPF 25%, NSIP 7%, DIP 2%, COP 4%), sarcoidosis 28%, hypersensitivity pneumonitis 10%, unclassifiable 6%, CTD-ILD 7%, drug-induced 3%; pneumoconiosis 2%, LAM 1%, PAP 1%, eosinophilic pneumonia 1%, others 1%; 3% familial forms. Relevant comorbidities were 23% GERD, 8% PH, 11% emphysema. Drug therapy comprised Azathioprine 12%, Prednisone 67%, NAC 6%, Pirfenidone 11%, Nintedanib 16%, Cyclophosphamide 4%, MTX 5%, MMF 2%, Rituximab 2%, Sirolimus 1%, long term oxygen 18%. Non-pharmacological therapies included physiotherapy 4%, NIV 4%, pulmonary rehabilitation 4%. Within 6 months prior to baseline, 50% were hospitalized, 91% of these due to ILD: diagnosis 70%, pneumonia 14% and acute exacerbations 17%. Survival differed significantly between subtypes (IPF the worst) and ILD severity (GAP 6-8 the worst).

**Conclusions:** The EXCITING-ILD registry mirrors diagnostic procedures, therapy and outcome of ILDs in Germany under clinical practice conditions. Substantial disease severity, a high rate of ILD associated hospitalizations and an underrepresentation of non-drug therapies warrants further attention.
Abstract No. 426

**Pulmonary surfactant as drug delivery system to target lung epithelium: new approach for the treatment of epithelial injury after bleomycin challenge**

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Idiopathic pulmonary fibrosis (IPF) is a progressively and ultimately fatal lung disease. Bleomycin-induced lung fibrosis model shows decreased activity of pulmonary surfactant (PS) followed by thickening of the septal wall and collagen deposition. PS is a complex mixture of lipids and proteins that reduce surface tension at the alveolar interface avoiding alveolar collapse. Chronic abnormally high surface tension contributes to fibrosis development by introducing mechanical stress. Besides, transforming growth factor beta 1 (TGF-beta1) is a cytokine related to fibroblast recruitment and fibrosis development. Therefore, TGF-beta1 blocking-based therapies have been developed in the last years. Interestingly, PFD is highly hydrophobic being thus a potential candidate for solubilization in lipids, such as PS lipids. Therefore, we aim to develop a therapy based in the combination of PS and PFD, benefiting from preventing mechanical stress and blocking TGF-beta1. Moreover, spread of PS in alveolar airspaces would help to locally deliver PFD, targeting alveolar epithelium, allowing us to reduce PFD dose and potentially avoid the side effects. Two experimental designs based on preventive (day 3 after bleomycin application) and therapeutic (day 7 after bleomycin) treatment of the animals with a combination of PS and PFD and the corresponding controls was performed. Functional alveolar dynamics (using a small animal ventilator Flexivent) and structure analysis by means of design-based stereology of the lungs were performed. In addition, surfactant proteins and inflammatory cytokines levels were also evaluated. Combination of PS and PFD showed better lung performance, with increased compliance and normalized elastance. Interestingly SP-C expression and other surfactant associated proteins such as TTF-1 and ABCA3, showed reversion to similar levels as in healthy controls, which suggest that the combination therapy is targeting alveolar epithelium, and especially alveolar epithelial type II cells (AE2C). In conclusion, combination therapy using PS as PFD vehicle increases beneficial effects of both components.
Abstract No. 427

Pirfenidone Ameliorates Pulmonary Fibrosis in Conditional Nedd4-2 Deficient Mice

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We developed a new model for diffuse parenchymal lung disease (DPLD) which is caused by a conditional deletion of Nedd4-2 and is characterized by patchy scarring of distal air spaces, restrictive lung physiology and signs of histological and radiological honeycombing. Pirfenidone is the first approved drug and the current gold standard for the treatment of IPF. In this study we tested the effects of pirfenidone on our mouse model.

To determine the effects of pirfenidone treatment on conditional Nedd4-2−/− mice, we induced Nedd4-2 deletion by doxycycline at the age of 4 weeks and started oral administration of 0.5% (w/w) pirfenidone in the chow or control diet 2 months after start of doxycycline induction. One month after treatment we performed inflammatory cell counts and measured cytokine levels of KC, IL-13 and IL-1β in the BAL as well as levels of TGFβ in lung homogenates of treated and untreated conditional Nedd4-2−/− mice and littermate controls. Furthermore, we determined lung mechanics by pulmonary function testing and quantified the volume of fibrotic lesion by micro-CT imaging in paraffin embedded lungs.

Our results demonstrate that mice treated with pirfenidone show improved lung compliance and reduced inflammatory parameters compared to untreated Nedd4-2−/− mice. Micro-CT imaging of paraffin embedded lung of treated and untreated conditional Nedd4-2−/− mice showed a reduction in volume of fibrotic lesions of the pirfenidone treated group. Furthermore, levels of active TGFβ were reduced in lungs of pirfenidone treated Nedd4-2−/− mice.

Taken together we observed that pirfenidone has a positive effect on disease progression of DPLD in conditional Nedd4-2−/− mice comparable to the therapeutic effects in patients. This underlines the value of this mouse model for preclinical testing, development and evaluation of novel therapeutic strategies in the treatment of DPLD.
Abstract No. 428

**B cell mediated autoimmunity in idiopathic pulmonary fibrosis**

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IPF is currently an incurable disease with limited understanding of its molecular pathophysiology. Diagnosis of IPF includes exclusion of autoimmunity based on a likely very incomplete panel of known autoantigens. We previously identified an unexpected high prevalence of MZB1 positive antibody-secreting plasma B cells in IPF tissues. MZB1 levels correlated positively with tissue IgG and negatively with lung function parameters, indicating a common involvement of antibody mediated autoimmunity in IPF (Schiller et al; Am J Respir Crit Care Med. 2017).

Experimental evidence in mice suggests that T cells targeting a single lung-specific autoantigen are sufficient to induce full blown and irreversible fibrosis (Shum et al, Sci Transl Med 2013). It is thus conceivable that (1) the presence of autoantibodies and autoreactive T cells may cause or at least perpetuate many IPF cases and (2) that identification of unknown autoantigens may serve as a powerful tool for patient stratification and future immunotherapy of IPF.

Here, we present an unbiased mass spectrometry (MS)-based autoantigen discovery platform based on patient plasma antibody capture and immunoprecipitation of a pooled protein extract derived from lung explants of endstage ILD patients (n=40; BioArchive). In a pilot experiment we analyzed antibody-antigen complexes from IPF (n=10) and CTD-ILD (n=10) patients, as well as healthy controls (n=10). Proof of concept was successfully reached using CTD-ILD cases clinically characterized to have Scl-70 autoantibodies. In comparison to healthy controls, we were able to correctly identify Scl-70 positive patients with 100% accuracy and specificity (n=5). Furthermore, the IPF cohort revealed several putative novel IPF autoantigens that were detected in up to 4 out of 10 IPF cases and none of the CTD-ILD cases. Future scaling of this pilot experiment to large patient cohorts and correlation of autoantibody profiles with clinical phenotyping will shed light on the involvement of humoral autoimmunity in IPF.
Abstract No. 429

The clinical impact of daily home spirometry and activity monitoring in progressive interstitial lung disease

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Background: In interstitial lung disease (ILD), forced vital capacity (FVC) is used as a prognostic factor for disease progression. Recently, it has been shown that daily home spirometry of FVC provides important additional information in idiopathic pulmonary fibrosis (IPF). Objective: The aim of this prospective study is to evaluate whether daily home spirometry can predict the course of FVC better than pulmonary function test after 3 and 6 months in patients with progressive ILD (IPF and non-IPF), potentially allowing for an earlier clinical decision making. Moreover, the daily activity will be compared with subjective well-being in the study cohort. Study design: Patients with progressive ILD (decline in FVC or clinical worsening) are asked to use daily home spirometry for 6 months. At baseline and after 3 months, patients further are monitored with an activity tracking device for 7 days. In addition, the subjective well-being is recorded in a diary. At baseline, after 3 and 6 months, complete pulmonary function testing including 6-MWD will be performed. Preliminary data: So far, 20 patients have been enrolled (IPF n=7, non-IPF n=13) from June 2017 until November 2017. After 3 months, patients’ adherence to daily home spirometry is 85.0±21.4% of the days and correlates significantly with baseline FVC (n=10; p=0.022). Although there is no statistical significant difference concerning FVC, DLCO and age between IPF and non-IPF, 6MWD (p=0.04) and steps per day (p=0.019) are significantly reduced in IPF compared to non-IPF (n=14). Conclusion: Our study aims to analyze the impact of intensified home monitoring with daily spirometry and activity monitoring in progressive ILD. Ideally, home monitoring will help with clinical decision making in the future. This study is funded by the Friedrich-Baur-Stiftung, Ludwig-Maximilians-University Munich
Abstract No. 430

**Functional assay of the role of ABCA3 in phosphatidylcholine metabolism**

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**Background**

Locating on the limiting membrane of lamellar bodies (LBs) in type II alveolar cells, ATP-binding cassette sub-family A member 3 (ABCA3) is a crucial protein that transports lipids into LBs to form pulmonary surfactant. Mutations in the ABCA3 gene can impair the protein functionally and cause lung diseases in newborn, children and adults. The lack of functional assays is a major obstacle for investigating lipids metabolism in LBs.

**Methods**

Human influenza hemagglutinin-(HA-) tagged wild type and mutant ABCA3 proteins were stably expressed in lung A549 cells. We used metabolic incorporation of the choline analog propargylcholine (PCho) to label phosphatidylcholine. At the end of the experiment, the tagged alkynyl lipid was reacted with an azido fluorophore and visualized by confocal microscopy. Images were analysed by Image J and the volume and fluorescence intensity of PCho in ABCA3-HA-positive vesicles were quantified.

**Results**

PCho accumulated in the vesicles in a time and dose dependent manner. The volume of the vesicles remained unchanged. When we inhibited the ATPase of the transporter, the fluorescence intensities of the vesicles decreased. Treatments were not toxic for the cells, as shown by XTT assay. Analysis of the cells by mass spectrometry showed that the molecular species composition of phosphatidylcholine composed of choline or PCho were similar, indicating that metabolic labelling can be successfully used to study LB lipid metabolism.

**Outlook**

The results suggest PCho is transported into preformed ABCA3 containing vesicles. In further studies, same assays would be done in several disease-causing mutants of ABCA3, to clarify the functional deviations from wild type.
Abstract No. 431

Towards an in situ interactome of the pulmonary basement membrane

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Systems level insights into the complex interaction landscape of the ECM in situ has long been elusive but may be key to understand tissue (patho-)physiology. ECM proteins are typically large multi-domain proteins, offering highly distinct interaction specificities. The recent combination of chemical crosslinking with mass spectrometry (CX-MS) opens the way for domain resolved ECM interactomics in situ. In CX-MS, a bifunctional chemical cross-linker covalently couples lysine residues that are in close proximity (~1nm) in protein interaction interfaces. After enzymatic digestion of proteins, chemical crosslinks are identified by MS and used as direct proof of interaction interfaces and as distance restraint for protein complex topology modeling.

The basement membrane (BM) is an ancient multiprotein structure underneath epithelia with key roles in organ development and functionality. Pulmonary BM composition and organization in the lung is currently poorly characterized. In this study we use CX-MS to screen for novel BM interacting proteins in murine lungs, which were natively decellularized before chemical crosslinking to increase coverage of ECM proteins. We identified 292 lysine crosslinks between and within 49 ECM, secreted and transmembrane proteins. As a proof of concept our experiment validated 14 of the 32 possible pairwise combinations of laminin α β γ chains. Furthermore, we were able to identify 25 novel putative direct interactors of the pulmonary BM in situ, which we are currently validating using complementary methods.
Abstract No. 432

**Cellular and molecular characterization of the mesenchymal niche for bronchiolar epithelial progenitor cells after naphthalene injury in mice**

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**Objective:** We have previously shown that ASMCs constitute a mesenchymal niche for bronchiolar epithelial progenitor cells after naphthalene (NA) injury (Volckaert et al., 2011). Recent work by Peng et al. (2015), showed that Gli1-positive cells around the airway could also contribute to this niche. Using lineage tracing-based approach combined with gain/loss of function for Ctnnb1 and Fgf10 expression, we aim to better characterize the mesenchymal niche at the cellular and molecular level.

**Results:** Deletion of Ctnnb1 using the Acta2-CreERT2 transgenic line before and after NA injury delays epithelial regeneration while deletion only before injury does not impact epithelial repair. Expression of a stable form of Ctnnb1 in Acta2-positive cells after injury enhanced epithelial repair. Deletion of Fgf10 before and after NA injury also delayed epithelial regeneration. Deletion of Fgf10 before NA injury also lead to decreased epithelial repair and was associated with Wnt7b downregulation. Lineage tracing of Fgf10-positive cells (Fgf10CreERT2/+; Tomato^lox/+^) labeled before NA injury shows accumulation of RFP-positive Acta2-negative cells around the bronchi at day 3.

**Conclusions:** Our results establish a new paradigm for further experiments addressing the function of signaling pathways in the different components of the mesenchymal niche for bronchiolar epithelial progenitor cells.
Abstract No. 433

Role of regulatory T-cells (Tregs) in bacterial infection-induced lung fibrosis exacerbation in mice

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Idiopathic pulmonary fibrosis (IPF) is a diffuse parenchymal lung disease, which causes an irreversible loss of lung function with a median survival from 2 to 5 years after diagnosis. Some patients with IPF experience an acute worsening of their disease termed acute exacerbation of IPF (AE-IPF), which may be caused by bacterial and/or viral infections. In the current study, we characterized the role of regulatory T cells in infection induced fibrosis exacerbation in mice, employing S. pneumoniae as bacterial pathogen. We found that mice exposed to AdTGFβ1 to trigger pulmonary fibrosis responded with a strong expansion of the Treg compartment, which was further significantly expanded during S. pneumoniae-induced fibrosis exacerbation. Diphteria toxin induced depletion of Tregs immediately prior to infection of mice with S. pneumoniae further aggravated exacerbation of lung fibrosis, whereas IL-2 complex-induced Treg expansion attenuated infection-induced fibrosis exacerbation. This was accompanied by a strong decline in Th1/Th2 cytokine release in mice. Collectively, these findings demonstrate a regulatory role for Tregs in infection-induced fibrosis exacerbation, and their expansion offers a therapeutic benefit in mice.
Impaired BMP signaling in the neonatal lung undergoing postnatal injury

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Background: Neonatal chronic lung disease (CLD) is characterized by impaired alveolar and vascular development, leading to adverse pulmonary outcome including the development of pulmonary hypertension. Defining the role of central signaling pathways in CLD development could reveal future treatment targets. We therefore investigated the role of the bone morphogenetic protein receptor 2 (BMPR2) signaling pathway, critical for vascular development, in the context of neonatal CLD. We furthermore explored increase of BMPR2 signaling through FK506 for its potential to rescue BMP signaling and improve vasculogenesis in CLD.

Methods: To study BMP signaling in vivo, we employed a mouse model of CLD and mechanically ventilated 5-7 day old C57BL/6 mice for 2h and 8h with 40% oxygen (MV-O2) and compared them to non-ventilated controls undergoing hyperoxia (fiO2 0.4) and room air (fiO2 0.21) treatment (n=4/group). Protein expression of phospho Smad1/5/9 as well as ID-1, a downstream target of BMPR2, was measured in whole lung lysates by western-immunoblotting. In vitro, human fetal pulmonary endothelial cells (EC) were exposed to in vitro stretch or hyperoxia or both for 24h in the presence or absence of TGF-β (5 ng/ml). CD31 expression was subsequently assessed by qRT-PCR.

Results: MV-O2 for 8h significantly reduced phospho SMAD 1/5/9 and ID-1 protein expression in total lung lysates (p<0.01), paralleled by reduced lung VE-Cadherin expression and small vessel number. This effect could be attributed to stretch and differed from the effect of oxygen treatment alone. FK506 fully restored CD31 mRNA expression in mouse lungs undergoing ventilation with oxygen rich gas.

Conclusion: Modulating BMPR2 signaling in neonatal CLD might be a promising strategy to improve impaired vasculogenesis in the course of the disease with FK506 being a potential treatment option.
Abstract No. 435

**Monocyte-Centred Inflammatory Response Driving Pulmonary Injury in Neonatal Chronic Lung Disease**

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**Background and Rationale:** Inflammatory response comprising influx of neutrophils and monocytes defines the onset of injury upon mechanical ventilation (MV) with oxygen in the developing lung. Resulting in imbalanced growth factor signaling it finally leads to impaired alveolar and vascular development, hallmarks of neonatal chronic lung disease (nCLD). In order to unravel clinically relevant disease mechanisms and identify treatable traits, we addressed the role of specific inflammatory mediators in ventilated preterm infants and characterized the functional consequences in a pre-clinical mouse model of the disease.

**Methods and Results:** Transcriptome analysis revealed significant enrichments in pathways highlighting apoptosis and inflammation related genes associated in nCLD patients associated with the length of MV and oxygen treatment. Subsequent investigation of a comprehensive cytokine panel (Luminex® xMAP® technology) in cord blood specimen from nCLD patients confirmed high levels of monocytic chemotactic proteins. Detailed FACS analysis revealed an elevation of a pro-inflammatory monocyte subset (CD14++/CD16- and CD14++/CD16+ monocytes/μl) in blood samples derived from preterm infants when compared to term neonates early after birth. As transcripts for genes related to the immune-modulator vitamin D are deficient in cord blood from nCLD patients we studied its deficiency in unique pre-clinical mouse model of the disease. Here, vitamin D deficiency was associated with monocyte activation, i.e. TGF-β signaling in response to MV. In vitro, this led to disrupted PDGF-Rα signaling and impaired myofibroblast function.

**Conclusion:** Early stages of nCLD development induced by MV-O2 are characterized by a monocyte/macrophage centered immune response and the release of TGF-β. The role of vitamin D as a potential regulator of this response is promising but awaits further exploration.
Abstract No. 436

**A new humanized Mouse Model for Idiopathic Pulmonary Fibrosis (IPF)**

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**Introduction**

The bleomycin model is widely used for target discovery and treatment evaluation, but resembles tissue remodeling in IPF only poorly. We established a new humanized mouse model on the basis of human airway basal cells (hABC) derived from either IPF patients, nonUIP PF patients or healthy controls. Nintedanib was recently approved for the treatment of IPF. The in vivo influence of nintedanib was tested.

**Methods**

hABCs were harvested from IPF patients, nonUIP IPF patients and healthy volunteers by brushing central airways during a bronchoscopy. At day 0 a low dose of bleomycin was given intratracheally. Three days later hABCs were intratracheally administered. hABCs were transfected with a vector encoding for GFP and luciferase, detected by IVIS Imaging. After 21 days the lungs were harvested and used for immunohistochemistry, HE and trichrome staining. In addition, hydroxyproline levels were measured by a commercially available kit. In some experiments mice were treated with nintedanib.

**Results**

Mice challenged with bleomycin and hABCs derived from IPF patients showed severe pulmonary fibrosis, bronchialization of the alveolar compartment and the evolution of cystic lesions. Furthermore, we found metaplastic lesions and engraftment of hABCs was documented by bioluminescence. In contrast, mice challenged with bleomycin only or bleomycin + hABCs derived from healthy volunteers, we could hardly detect any fibrotic lesions. Ashcroft score and hydroxyproline levels were statistically significantly lower in nintedanib treated mice.

**Conclusion**

Our data strongly suggest a profibrotic role of hABCs in IPF. The model resembles IPF better than previously used standard models.
Abstract No. 437

FK506-Binding protein 11, a plasma cell-specific antibody folding catalyst, is increased in pulmonary fibrosis

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RATIONALE: We have recently identified FK506-binding protein 10 (FKBP10) as a profibrotic mediator in idiopathic pulmonary fibrosis (IPF). In this study, we sought to assess expression, localization, regulation and function of a related protein, FKBP11, in lung fibrosis.

METHODS: Expression of FKBP11 in lung tissue was analyzed in lungs from independent IPF cohorts using Western Blots and DNA-microarray data. Immunofluorescence of IPF sections was used to determine the cell type expressing FKBP11 and to quantify FKBP11-positive cells in IPF relative to donor and chronic obstructive pulmonary disease (COPD). Additionally, FKBP11 was assessed in a B-cell line, antibody-producing hybridoma cell lines, peripheral blood mononuclear cells (PBMCs) and A549 under conditions of endoplasmic reticulum stress (ER-stress). Finally, knockdown experiments and in vitro refolding of immunoglobulin G under addition of recombinant FKBP11 were performed to investigate the function of FKBP11.

RESULTS: All three IPF cohorts displayed increased FKBP11 expression relative to healthy controls. In agreement, immunofluorescent stainings in IPF sections showed elevated numbers of FKBP11-positive cells in contrast to healthy donor and COPD. Costainings with markers for the hematopoietic lineage demonstrated specific expression of FKBP11 in CD45-/CD20-/CD38+/CD27+/CD138+ plasma-cells producing mainly immunoglobulin A and G. Hybridoma cells displayed strong expression of FKBP11 and induction of ER stress in a B-cell line and A549, as well as B-cell to plasma-cell transdifferentiation in PBMCs resulted in upregulation of FKBP11. Knockdown of FKBP11 in hybridoma cells reduced cell viability. Finally, recombinant FKBP11 improved refolding rate and yield of immunoglobulin. Latter effects were inhibited by Tacrolimus.

CONCLUSIONS: The protein folding catalyst FKBP11 is increased in IPF, specifically localizes to plasma-cells in the lung, and is regulated by ER-stress. FKBP11 folds antibodies in vitro and is inhibited by Tacrolimus. These results argue for an important role of FKBP11 in plasma cell biology and support the autoimmune hypothesis in IPF.
Abstract No. 438

**MIRLET7D delivery as therapeutic approach against Idiopathic Pulmonary Fibrosis**

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**Objective:** Idiopathic Pulmonary Fibrosis (IPF) is a chronic, irreversible and highly lethal disease. We previously showed that the MiCEE complex mediated silencing of bi-directional expressed genes in lung epithelial and mesenchymal cells. However, the clinical relevance of these findings was not known.

**Results:** Here we show that reduced nuclear *MIRLET7D* levels in idiopathic pulmonary fibrosis (IPF) compromised epigenetic silencing mediated by the MiCEE complex. In addition, we found that reduced nuclear HDAC activity in IPF interferes with MiCEE-dependent H3K27 tri-methylation. Furthermore, we demonstrated that *MIRLET7D* gain-of-function *in vitro* reduced fibrotic hallmarks of human primary fibroblast.

**Conclusion:** We elucidated the clinical relevance of the MiCEE complex within a highly lethal pulmonary disease. Our results together highlight the potential of *MIRLET7D* as basis for therapeutic approaches against IPF.
Abstract No. 439

Orchestrating cholesterol homeostasis – The novel role of surfactant protein C

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Lung surfactant is a complex mixture of lipids and proteins, which reduces surface tension at the air-water alveolar interface, decreasing the work of breathing and avoiding alveolar collapse. Several mutations in the SP-C gene have been associated with the development of familial forms of lung fibrosis. SP-C has been described to counter-act cholesterol deleterious effect on surfactant film stability at the interface. SP-C KO mice breathe and live normally, but they show higher susceptibility to chronic lung diseases and induced fibrosis with age. Numbers of cells in BAL are elevated in SP-C deficient mice, while electron microscopic images showed foamy alveolar macrophages (AM) loaded with lamellar body (LB)-like structures. At an age of 30 weeks solid electron dense crystals were observed to start appearing in AM. Further preliminary studies could correlate these findings with significant downregulation of Abcg1, a cholesterol efflux transporter. In order to obtain a broader overview about the aberrant lipid metabolism and to cover other potentially dysregulated metabolic pathways in these cells, additional transcriptome analysis was assessed. Consecutive gene expression analysis in lung tissue revealed significant upregulation of Abca1, which may compensate for impaired AM function. To confirm whether SP-C may have an effect on lipid metabolism and degradation in AM, we performed in vitro endocytosis and gene expression studies using the murine alveolar macrophage cell line MH-S. Addition of cholesterol to surfactant lipids increased the uptake ratio of these cells and differentially regulated a set of genes involved in cholesterol transport, regulation and metabolism, such as Abca1, Abcg1, Npc2, Cd36, Pparg, Lal and Dhcr24. SP-C in combination with cholesterol in surfactant membranes further activates genes related to cholesterol transport and regulation. In conclusion, SP-C seems to play an important role not only in surfactant activity and alveolar dynamics, but also in lipid metabolism in alveolar macrophages.
Abstract No. 440

Pro-fibrotic role of Histone Deacetylase 9 isoforms in pulmonary fibrosis

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Idiopathic Pulmonary Fibrosis (IPF) is a progressive and fatal interstitial pulmonary disease with no proven drug therapy. The disease is characterized by the presence of phenotypically altered fibroblasts that exhibit hyper-proliferation, apoptotic resistance, increased migration and trans-differentiation. Recently, the role of epigenetic modifiers such as histone deacetylases (HDACs, Class IIa) has been identified in the pathophysiology of many fatal diseases such as cancer and cardiovascular diseases. However, the role of class IIa HDACs in the progression of IPF and specifically in driving IPF-fibroblast phenotype is yet to be investigated. We examined fibrotic lungs and lung fibroblasts isolated from patients with IPF and comparative controls and assessed the expression of class-II HDAC family members (HDAC4, HDAC5, HDAC6, HDAC7, HDAC9 and its alternatively spliced isoform HDRP and HDAC10). We found that HDRP expression is up-regulated exclusively in fibroblasts isolated from IPF patients compared to fibroblasts isolated from healthy controls/donors. Assessing the pro-fibrotic mediators that can regulate the expression of HDACs, we observed that the TGF-β, leads to up regulation of both HDAC9 and HDRP expression, However the expression of remaining class IIa HDACs (HDAC4, HDAC5, HDAC6, HDAC7, HDAC9 and its alternatively spliced isoform HDRP and HDAC10). We found that HDRP expression is up-regulated exclusively in fibroblasts isolated from IPF patients compared to fibroblasts isolated from healthy controls/donors. Assessing the pro-fibrotic mediators that can regulate the expression of HDACs, we observed that the TGF-β, leads to up regulation of both HDAC9 and HDRP expression, However the expression of remaining class IIa HDACs (HDAC4, HDAC5 and HDAC7) remain unaltered. Our functional data shows that the overexpression of HDRP and HDAC9 leads to fibroblastic myofibroblast trans-differentiation and apoptosis-resistance in donor Fibroblasts. In addition, down-regulation of HDRP using siRNA not only reverses the myofibroblast phenotype of the TGF-β stimulated donor fibroblasts as well as of the IPF fibroblasts but also increases the apoptosis in IPF fibroblasts. The mass spectrometry analysis highlights diverse functions for HDAC9 and HDRP including the regulation of Focal adhesion signalling. We additionally confirmed the interactions with the Co- immunoprecipitation. Our primary data suggests that TGF-β mediated regulation of HDRP and HDAC9 modulates lung fibroblast phenotypes and hence contributing to the fibrotic pathology in the pathogenesis of IPF.
Abstract No. 441

Voluntary Activity Alleviates Obesity-Induced Structural Changes of the Lung

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Obesity is a growing pandemic health problem and linked to a wide range of respiratory conditions including asthma and COPD. Physical exercise beneficially affects lung function; however, it is unclear whether there is a structural correlate to this. We hypothesize that voluntary activity improves lung mechanics and diminishes pulmonary structural changes in obese mice. Male C57BL/6N mice were fed control diet (CD) or high fat diet (HFD) and were housed in cages with or without running wheels resulting in the experimental groups CD, CD-active, HFD and HFD-active. Body weight, food consumption and running distance were weekly recorded. After 30 weeks, lung mechanic measurements were performed and left lungs were processed and analyzed according to design-based stereological standards. HFD induced significantly higher body weights due to increased calorie intake in comparison to CD. Voluntary activity resulted in higher calorie consumption irrespective of the diet, what however did not affect weight gain. Furthermore, HFD decreased weekly running distances. Diet and activity exerted only minor effects on lung mechanics including an overall reduced elastance induced by voluntary activity and an overall increased inspiratory capacity in HFD-fed mice, particularly for active groups. Static compliance was unaltered. Left lung volumes as well as volumes of alveolar air space and septa were significantly increased in response to HFD. This was accompanied by higher septal surface and thickness. The volumes of cellular and extracellular septal components were enhanced proportionally. Remarkably, voluntary activity alleviated HFD-related changes in lung and alveolar air volume, septal surface and epithelial, endothelial and interstitial cell volumes. Moreover, septal volume and thickness as well as septal ECM volumes were normalized to control levels. We conclude that voluntary activity, even if ineffective against weight gain, is a potent intervention strategy against obesity-related pulmonary structural changes, what may contribute to exercise-induced improvement of lung function.
Abstract No. 442

LRP1 - a master regulator of the contractile activity of myofibroblasts

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Expansion of myofibroblast population tightly correlates with maladaptive tissue repair. Over-activation of the transforming growth factor (TGF) β signaling pathway supports this process by inducing differentiation of fibroblasts to myofibroblasts. As the TGF-β signaling pathway is known to be regulated by receptor low density lipoprotein-receptor related protein (LRP) 1, we hypothesized that LRP1 controls a phenotypic switch of fibroblasts to myofibroblasts. Our data demonstrate that depletion of LRP1 in human lung fibroblasts associates with the increased mRNA and protein levels of α-smooth muscle actin (α-SMA). Interestingly, activation of the α-SMA alternative promotor was responsible for the generation of α-SMA mRNA in LRP1-depleted cells. Furthermore, LRP1-depletion correlated with the increased activity of SMAD3 and c-Jun N-terminal kinase (JNK) 1/2, the downstream effectors of the canonical and the non-canonical TGF-β signaling pathway. Silencing of JNK 1/2 and SMAD3 suppressed expression of α-SMA induced by LRP1 knock-down. Moreover, LRP1 loss stimulated lung fibroblast-mediated collagen gel contraction. In summary, our results demonstrate that downregulation of LRP1 expression enhances the TGF-β signaling in human lung fibroblasts and thus promotes the generation of the contractile phenotype. Thus, restoration of LRP1 expression might suppress excessive myofibroblast accumulation and in consequence abnormal wound healing.
Abstract No. 443

**Associations between comorbidities, their treatment and survival in patients with interstitial lung diseases – a claims data analysis**

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**Background:** Interstitial lung diseases (ILDs) are associated with a high burden of disease. However, data on the prognostic impact of comorbidities and comorbidity-related pharmaceutical treatments in patients with various ILDs are sparse.

**Methods:** Using longitudinal claims data from a German Statutory Health Insurance Fund, we assessed comorbidity in ILD subtypes and associated drug treatments. Baseline comorbidity was assessed via the Elixhauser Comorbidity Index, which was amended by ILD-relevant conditions. Drug treatment was assessed on the substance level using ATC-code drugs prescribed at the time of ILD diagnosis. Subsequently, impact factors on survival in the total ILD cohort and distinct ILD subtypes were identified with multivariate Cox models via LASSO selection, and respective comorbidomes were created.

**Results:** In 36,821 patients with ILDs, chronic obstructive pulmonary disease (COPD), arterial hypertension and ischaemic heart disease (IHD) were the most prevalent comorbidities. The majority of patients with cardiovascular diseases received pharmaceutical treatment whereas, in other relevant comorbidities, treatment quotas were low (COPD 46%, gastro-oesophageal reflux disease / GERD 65%). Comorbidities had a clinically meaningful detrimental effect on survival that was more pronounced in the case of untreated conditions (e.g. hazard ratios for IHD 0.97 (treated) vs. 1.33 (untreated)). Moreover, comorbidity impact varied substantially between distinct subtypes.

**Conclusions:** Our analyses suggest that comorbid conditions and their treatment profile significantly affect mortality in various ILDs. Therefore, comprehensive comorbidity assessment and management is important in any ILD.
Abstract No. 444

**Surfactant protein C (SP-C) deficiency leads to a combination of over-distended alveolar and ductal spaces with focal fibrotic remodeling of the lung.**

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Lung surfactant is a complex mixture of lipids and proteins, which reduces surface tension at the air-water alveolar interface, decreasing the work of breathing and avoiding alveolar collapse. SP-C is a small hydrophobic protein providing stability of the lung surfactant film allowing low surface tension. Mice which lack SP-C breathe and live normally, but they show higher susceptibility to chronic lung injury and fibrotic remodeling related to lung aging. We chose this animal model to simulate the development of the familial form of lung fibrosis associated with mutations in the SP-C gene in humans. SP-C deficient mice were investigated at 6 different ages starting with the age of 10 up to 60 weeks, every 10 weeks. Analysis of lung mechanics was performed by connecting the mice to a small animal mechanical ventilation system. After fixing the lungs by vascular perfusion a systematic uniform random sampling was performed and lung structure was analyzed by stereology. Lung mechanics showed a tendency to lower elastance values through time and normalized compliance. However, tissue hysteresivity increased along septal wall thickness and lung function, by means of oxygen saturation, was reduced as well as alveolar surface area. A detailed structural analysis of the lung showed re-organization of air-spaces in the SP-C deficient mice characterized by a shift of airspaces from alveoli to ducts. Lack of SP-C increased the heterogeneity in alveolar volumes combined with over-distended ductal air spaces and increasing septal wall thickness with age. In conclusion, this structure-function study shows early alterations of air spaces turning towards a fibrotic tissue remodeling at older ages in the absence of SP-C.
Abstract No. 445

**Secreted Frizzled Related Protein 1 (SFRP1) is an early marker of lung fibrosis and acts as a novel regulator of the RhoA/Rock1 pathway**

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Idiopathic pulmonary fibrosis (IPF) is a progressive and fatal lung disease without an effective cure. The fibroblasts' activation, invasion and transdifferentiation into myofibroblasts are hallmarks of active lung fibrogenesis. In a time-resolved analysis of lung remodeling upon Bleomycin injury, we quantified the protein expression of SFRP1 from whole lung tissues on day 3, 7, 14, 28 and 56 by immunoblotting, and observed and increase of SFRP1 protein levels from day 3 on with peaks on day 14 and day 28. We found that expression of SFRP1 was highly specific for fibroblasts. *In vitro* cultured mouse and human fibroblasts strongly expressed SFRP1, but these cells downregulated SFRP1 expression upon their transdifferentiation into myofibroblasts upon treatment with the profibrotic master regulator Transforming growth factor beta 1 (TGFβ1). Single cell suspensions from whole lung tissues of day 14 healthy and Bleomycin instilled mice were analyzed via drop-seq single cell RNA-sequencing, which revealed a SFRP1 expressing subpopulation of pulmonary fibroblasts, which are clearly distinct from α-smooth muscle actin expressing (myo)fibroblasts. In-depth immunohistochemical analyses of fibrotic mouse lungs corroborated this finding further. To mimic early fibrotic conditions *ex vivo*, Mouse precision-cut lung slices (PCLS) were stimulated by TGFβ1 for 72 hours, which revealed an upregulation of SFRP1 on protein levels. Depletion of SFRP1 by siRNA mediated gene silencing did not affect the canonical Wnt pathway, but caused a significant reduction of RhoA and Rock1 protein levels, both of which are members of the non-canonical Wnt pathway. Consequently, SFRP1 depleted primary human fibroblasts revealed striking morphological and actin related cytoskeletal changes, with small-elongated cell bodies and a reduction of stress fibers. In conclusion, SFRP1, expressed by a distinct subpopulation of fibroblasts in fibrotic lungs, might act as a novel regulator of the non-canonical Wnt-pathway along the Rho-Rock1 axis interfering with cellular morphological changes.
Cooperation of ER stress and viral infection in fibrosis development

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ER stress and viral infections are believed to play an important role in Idiopathic Lung fibrosis (IPF) development and are considered as possible trigger mechanisms for acute exacerbations. Thus we hypothesize that epithelial ER stress affects the cellular response to viral infection and replication and aggravates the fibrotic process. To that end, we assessed how overexpression of single ER stress markers XBP1, ATF6 and CHOP in alveolar epithelial cells affected the infection process. We used PCLS from transgenic mice conditionally overexpressing each of three ER stress markers. After transgene induction PCLS were infected with influenza virus (strain A/Puerto Rico/8/1934 H1N1). Forty-eight hours later we evaluated the efficiency of infection, viral replication and epithelial apoptosis by FACS and immunofluorescence staining. While all ER stress markers were successfully induced, only overexpression of XBP1 and CHOP dramatically increased the number of infected epithelial cells. CHOP overexpression alone led to an increase in epithelial apoptosis, and virus infection led to dramatic increase in the number of apoptotic epithelial cells. ATF6 overexpression was inconsequential to the epithelial cell infection and survival. Further experiments will assess the cooperation of ER stress and viral infection and their role in the pathogenesis of lung fibrosis in vivo in mouse models of ER stress.
Abstract No. 447

Immunoproteasome function in pulmonary fibrosis

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Immunoproteasomes are the main type of protein degrading proteasomes in immune cells. They have been shown to shape adaptive immune responses by generating epitopes for MHC I antigen presentation. We have previously demonstrated the impact of the immunoproteasome subunit LMP7 on polarization of alveolar macrophages (AM) towards the profibrotic M2 phenotype in vitro indicating that immunoproteasomes also participate in shaping innate immune responses. We performed a multiparametric analysis of human and murine samples including Western blot, proteasome activity assays, immunohistochemistry as well as RNA expression analysis via qRT PCR. To investigate proteasome activity and expression in pulmonary fibrosis in vivo, we induced lung fibrosis in C57BL/6 mice by intratracheal instillation of bleomycin. Immunoproteasome expression was upregulated in lungs after 7, 14, and 56 days. Explanted lungs were obtained from 13 patients with endstage IPF and compared to 10 donor samples. In Western blot analysis of whole tissue, LMP7 immunoproteasome protein expression was significantly increased. Immunohistochemical analysis of human and murine lungs demonstrated a significant expression of the LMP2 immunoproteasome subunit not only in alveolar macrophages but also in the interstitium and epithelium of the fibrotic lung. These data suggest that immunoproteasome upregulation is an integral part of fibrotic remodeling of the lung. We also observed higher mRNA levels of immunoproteasome subunits LMP2 and MECL-1 in peripheral blood monocytes (PBMCs), but not in BAL samples of IPF patients.

We here provide evidence that the immunoproteasome is upregulated in both the bleomycin mouse model of lung fibrosis and in human pulmonary fibrosis.

Collecting PBMCs and matching BAL from IPF patients within the CPC-M Bioarchive, as well as validating new specific immunoproteasome inhibitors will be an important approach for better understanding the role of immunoproteasomes in the pathogenesis of pulmonary fibrosis with possible therapeutic consequences.
Abstract No. 448

Analysis of Emilin-2 functions in lung injury and repair

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Cell-matrix interactions play a pivotal role in lung disease and regeneration. We previously identified the extracellular matrix protein Emilin-2 as a novel provisional matrix component in lung injury and repair in the bleomycin injury mouse model. Emilin-2 can be part of ECM microfibrils and was shown to regulate cell survival and Wnt-signaling. In the context of lung injury, repair and fibrosis, the function of Emilin-2 is unknown. In this study we challenged Emilin-2 knockout mice and their wild type littermates with bleomycin injury and found reduced survival of knockout animals. Lung function parameters were not altered, indicating that the reduced survival in the inflammatory phase at day 7-10 after injury might be due to an immunomodulatory function of Emilin-2.

To address this hypothesis, we used high-throughput single cell RNA-sequencing (scRNA-seq) to determine the protein’s cellular origin and the cell types that respond to Emilin-2 expression. Furthermore, we used mass spectrometry driven proteomics and immunoassays to determine tissue level effects of the Emilin-2 knock out on ECM composition and cytokine profiles upon lung injury. We performed scRNA-seq from ~20.000 cells from whole lung single cell suspension derived from Emilin-2 knock out and wilde type animals 14 days after oropharyngeal instillation of bleomycin (3U/kg) or PBS. Our preliminary data shows that Emilin-2 expression in the lung is confined to several distinct monocyte/macrophage populations. Upregulation of Emilin-2 is also guided by these monocytes and macrophages. Ongoing experiments that will be reported at the meeting address the role of Emilin-2 in modulating the immune response that ensues upon acute lung injury.
Abstract No. 449

Quality of life assessment in ILD – a comparison of EQ-5D with the disease-specific K-bild

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Background King’s Brief Interstitial Lung Disease questionnaire (K-BILD), a disease-specific quality of life questionnaire (QoL) for individuals with interstitial lung disease (ILD) has not been applied in a German setting yet. Thus, a comparison of K-BILD with the well-established EQ-5D-5L could show its usability in clinical settings.

Methods We calculated mean of EQ-5D experience-based values (ebvs), visual analog scale (VAS) and K-BILD; additionally their correlation in total score and in different domains for 229 patients with different ILD subtypes from the longitudinal observational HILDA study. Furthermore potential predictors (age, sex, education, employment, smoking status, clinic, ILD subtype, FVC percentage of predicted value (FVC%), DLCO percentage of predicted value, and sum of comorbidities (primary analysis) or distinct comorbidities (secondary analysis) for HRQoL were investigated with linear regression models.

Results Means were as follows EQ-5D ebvs 0.66 (SD 0.17), VAS 61.4 (SD 19.1) and K-BILD 53.6 (SD 11.7).

EQ-5D and K-BILD showed moderate positive correlations (EQ-5D- ebvs vs. K-BILD 0.71; VAS vs. K-BILD 0.55). The “breathlessness” (K-BILD) domain highly correlated with “usual activities” (-0.69) and “mobility” domain (-0.65) (EQ-5D). Other domains showed moderate correlations. In the primary analysis higher FVC% was associated with higher QoL (EQ-5D index 0.0028 p=0.0001; VAS 0.19 p=0.0149; K-BILD 0.15 p=0.0015) whereas comorbidity burden with lower QoL (EQ-5D index -0.032 p=0.0001; VAS: -2.72 p=0.0011; K-BILD -1.51 p=0.0022). In the secondary analysis FVC% showed similar results (0.0023 p=0.0007; 0.2 p=0.0189; 0.16 p=0.0022). Arterial hypertension was with a lower EQ-5D ebvs and “breathlessness” scores associated (-0.05 p=0.0441; -6.85 p=0.0173) and Depression with lower K-BILD (-17.4 p=0.0029). Other factors had no significant influence.

Conclusions K-BILD and EQ-5D score reveal similar QoL trends and are sensible to the same disease-related factors. K-BILD reacts more sensitively to ILD-specific aspects of QoL rendering it a valuable complementary measure to EQ-5D-5L.
Impact of early nutrition on severity of BPD

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Objective: Bronchopulmonary dysplasia (BPD) is the chronic lung disease of the premature infant (PI) with lifelong pulmonary, somatic and psychomotor consequences based on the arrest of the progress of lung development in the saccular stage. Therapies are still limited and recent progress in the pulmonary outcome is mainly due to changes in clinical routine including ventilation, surfactant application and vitamin A supplementation. So far, the impact of nutrition on the incidence and severity of the BPD has not been studied except for the positive effect of breast milk and early full enteral nutrition.

Methods: PI with a gestational age (GA) ≤ 32+0 weeks and birth weight (BW) <1000 g who were born at our Level 3 perinatal center Gießen between 02/06 and 01/17 were retrospectively analysed for nutrition. The definition of Jobe and Bancalari was used to assess the severity of BPD. The parameters were qualitatively and quantitatively evaluated, partially correlated with the severity of BPD and controlled for GA, BW, sex, single vs. multiple births and antenatal steroids.

Results: A total of 207 PI (mean BW 800 ± 166 g; mean GA 26.6 ± 1.91 weeks) were analysed: nutrition of 144 (69.6 %) patients with no or mild BPD was compared with 63 (30.4 %) patients with moderate or severe BPD. A higher overall nutritional supply until day 14 led to a statistically significant decrease in the severity of BPD. Particularly carbohydrates (r= -0.146, p= 0.038) and caloric intake (r= -0.142, p= 0.044) showed a statistically significant result. In contrast, a high oral vitamin A supply during the first 14 days of life did not significantly decrease the severity.

Conclusion: Early high caloric nutrition has a positive effect on the severity of BPD. Future animal and prospective cohort studies should aim to clarify the impact of selected nutrients.
Abstract No. 451

Transbronchial cryobiopsies for diagnosing interstitial lung disease: real life experience from a tertiary referral center for ILDs

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Introduction Transbronchial cryobiopsy (cTBB) may offer an alternative to surgical lung biopsy (SLB) for histopathological diagnosis of ILDs. However, real life experience is limited. We therefore aimed to evaluate the value of cTBB under real life conditions of a tertiary care center for ILDs.

Methods Patients who received cTBB as workup for suspected ILD were evaluated retrospectively. Demographics, intervention related aspects were assessed and interpreted in the context of a multidisciplinary team discussion (MDT).

Results From 10/2015-01/2017 109 patients (mean age 64 years, 72 male, 38% never smokers) received cTBB for diagnosing ILD. Mean FVC was 77% (range (r) 41-131%pred.), mean DLCO 51% (r 20-86% pred.) On average, 4 samples were taken, in 67 % from 2 lobes Mean sample diameter was 5mm (r 2 -12 mm). Complications were pneumothorax in 12% (all requiring chest tube) And moderate bleeding in 28%. Acute exacerbations did not occure. In histopathologic assessment, specimen were judged as diagnostic in 73% and the following patterns were described: NSIP (22), UIP (11), consistent with HP (7), smoking-related ILD (11), unspecific fibrosis (29). In the context of MDT final diagnoses were possible in 84 %. with HP (3), idiopathic NSIP (8), drug toxicity (10), IPF (4), CDT-ILD (6), smoking related ILD (5) and other (5). SLB was indicated in 13 cases, but unfortunately was not feasible in 5 cases, 3 patients were excluded because of their comorbidities and 2 patients declined. Since the introduction of the guideline in 2011, the rate of surgical lung biopsies of the newly diagnosed ILD patients dropped from 5.69% to 3.47%.

Conclusions In the real world setting, cTBB has a meaningful diagnostic value in the context of a multidisciplinary team and may enable histopathological assessment in more advanced disease.
Abstract No. 452

Phosphoproteomics reveals the signaling landscape of lung fibroblast rigidity sensing

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Cells can translate the mechanical properties of the extracellular matrix (ECM) into biochemical signals. Mechanosensitive signaling pathways are important in the pathophysiology of fibrotic diseases and cancer, which have in common that the composition of the ECM and its mechanical properties are altered in disease progression. In this study, we aimed at a systematic and unbiased exploration of molecular changes during mechanosensing using mass spectrometry driven phosphoproteomics.

We analyzed the phosphorylation landscape of two primary human and one mouse lung fibroblast lines, seeded for 3h on fibronectin coated PDMS substrates with varying elastic moduli (0.2, 0.5, 2, 8, 16, and 32 kPa), at a depth of >10,000 phosphosites quantified. This resulted in identification of significantly regulated phosphorylation sites (n=1202, n=1920 and n=4419), enriched for annotations such as RNA binding proteins, spliceosomal components, transcriptional regulators, endosomal components and cytoskeletal proteins. Two-way ANOVA and hierarchical clustering analysis was used to distinguish cell line specific phosphorylation events from common changes that occur in all three cell lines upon changes in ECM substrate rigidity. To functionally interrogate phosphorylation switches in mechanosensing we established a stable and inducible Cas9 expressing lung fibroblast line that will be used for the generation of knock out and phosphosite-mutants of critical nodes in the signaling network. Furthermore, we are currently using mass spectrometry to analyze the accompanying alterations in subcellular localization of proteins, and how these are regulated by the phosphorylation sites discovered in this study.
Programmed Cell Removal of Epithelial Cells in Lung Fibrosis

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Idiopathic lung fibrosis is a progressive, dismal lung disease characterized by extensive alveolar epithelial injury and fibroblast proliferation. Our study aims at characterizing a novel cellular surveillance mechanism called Programmed Cell Removal (PrCR) meant to ensure the removal of apoptotic cells by macrophage phagocytosis in IPF. PrCR relies on the balance between two innate immune checkpoint molecules, CD47 (“don’t eat me” signal) and soluble Calreticulin (“eat-me” signal) expressed by epithelial cells, which interact with their cognate receptors on the surface of macrophages. We demonstrate that in the IPF lung there is a significant decrease in epithelial CD47, but PrCR is abolished by a concomitant decrease in macrophage bound CALR. However, in the bleomycin model of lung fibrosis in which the fibrotic phenotype is transitory, the decrease in epithelial CD47 is paralleled by an increase in CALR⁺ macrophages, suggesting that PrCR is functional and might be a significant contributor to the resolution phase. Additionally we demonstrate that ER stress, the main culprit in epithelial injury in lung fibrosis, efficiently induces PrCR in vitro and ex vivo. In conclusion our data suggests that PrCR is a viable therapeutic target to moderate the evolution of the fibrotic process.
Abstract No. 454

**Notch1 Inhibition Restores Alveolar Epithelial Differentiation and Surface Tension and Limits Matrix Deposition in Lung Fibrosis**

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Alveolar epithelial cell type II (AEC2) injury underlies idiopathic pulmonary fibrosis (IPF), a dreadful disease. Here we show increased Notch1 signaling in IPF AEC2s and in the bleomycin model of lung fibrosis, causing enhanced proliferation and de-differentiation of AEC2s. Transgenic overexpression of Notch1 intracellular domain leads to overt lung fibrosis, AECII proliferation and defective surfactant protein (SP)-B/C processing. We show that in IPF patients this increase in surface tension can be efficiently rescued by mature SP-B supplementation. HRC-CT data show increased alveolar collapse in the fibrotic areas, most likely leading to AEC injury. Furthermore Notch signaling inhibition by the γ-secretase inhibitor DAPT in human IPF precision lung cut slices improves surfactant processing, expands the surfactant secretory machinery and reduces collagen expression. Thus we conclude that in IPF Notch1 signaling promotes a vicious cycle of injury and repair leading to lung fibrosis and that targeting Notch1 represents a viable therapeutic option for IPF patients.
Abstract No. 455

**Predictive factors of drug treatment in children with ILD: First insights from the chILD-EU Registry**

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OBJECTIVES: Childhood Interstitial lung disease (chILD) is an umbrella term for more than 200 rare entities for which evidence on medical treatment regimens, healthcare utilization and clinical outcomes are broadly lacking. We aimed to evaluate current drug utilization.

METHODS: The ongoing chILD-EU registry was initiated in 2012 and currently includes children with ILD from 7 European countries. Next to baseline characteristics, the 5 most frequent medications taken in the 3 months prior and up to study baseline were analysed via logistic regression models adjusted for sex, age, country, disease severity and diagnosis.

RESULTS: Among the 336 patients (56.3% male) median age was 3.9 years (IQR 0.8, 10.8 years) with a median disease severity of 3 on the FAN 5 point scale. 282 (83.9%) patients had a final diagnosis of 72 different diseases on subcategory level. Within the 3 months prior to baseline or at baseline all patients received at least one kind drug treatment, 35.1% received systemic steroids, 27.1% long term macrolides, 22.9% inhaled steroids, 22.9% antibiotics, and 20.8% hydroxychloroquine. Regression models examining whether a specific treatment was received (yes/no) showed that diagnosis (reference: surfactant disorder (A4); sign odds ratio (OR): ranged between 0.15 and 15.5 was usually significantly associated with receiving a medication. Disease severity was also significantly association with receiving a medication (reference: asymptomatic; sign OR: 3.9 to 20.8. Country specific influences on treatment regime (using Germany as the reference country) were seen regarding long term macrolide therapy (Poland OR: 0.16, UK OR 2.5), hydroxychloroquine therapy (UK OR: 3.6), and antibiotic therapy (UK OR: 0.3).

CONCLUSIONS: Corticosteroids are the most frequently used treatment. Complex longitudinal analyses of chILD-EU are needed to determine whether conclusions can be made regarding the association medications with treatment success, mortality or quality of life outcomes due to the “registry” nature of the data.
Reduced low-density lipoprotein receptor-related protein 1 expression is a signature of organ fibrosis – contribution to the imbalanced lung repair

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Imbalanced lipid homeostasis, alveolar epithelium injury and excessive deposition of extracellular matrix (ECM) are hallmarks of idiopathic pulmonary fibrosis (IPF). Considering that low-density lipoprotein receptor-related protein (LRP) 1 is a master regulator of lipid metabolism and ECM turnover, we hypothesized that LRP1 function may be impaired in IPF. LRP1 expression was reduced in alveolar epithelium and lung fibroblasts isolated from IPF patients. Interestingly, LRP1 levels in mice subjected to bleomycin-induced pulmonary fibrosis were decreased during the fibrotic phase and then partially restored at the onset of fibrosis resolution. Furthermore, LRP1 expression declined during progression of liver fibrosis in humans and in two distinct transgenic mouse models of that disease. Natural antisense transcript specific to LRP1 (LRP1-NAT) was induced in IPF and in bleomycin-treated lungs implying that LRP1 expression in fibrosis is repressed by cis-acting long non-coding RNA. Transfection of siRNA directed against LRP1-NAT enhanced LRP1 expression and decreased collagen levels in human lung fibroblast isolated from IPF patients. Inducible, cell-specific Lrp1 gene knock-out in fibroblasts or alveolar epithelium markedly aggravated bleomycin-induced pulmonary fibrosis in mice as evident by increased mortality, reduced lung compliance and exaggerated production of ECM proteins. In conclusion, these results indicate that perturbed LRP1 expression is a common attribute of fibrotic processes in various organs and that LRP1 is critical for proper lung regeneration after injury. Therefore, reconstitution of LRP1 expression could offer a potential therapeutic option for the treatment of pulmonary fibrosis.
Abstract No. 457

Immunization of mice with human AT1R generates AT1R-activating antibodies and induces a SSc-like disease

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Systemic sclerosis (SSc) is a complex connective tissue disease which is characterized by autoimmunity, vasculopathy and fibrosis. Recent studies have suggested that autoantibodies (aab) against angiotensin II receptor I (AT1R) might play a pathogenic role in the development of SSc, which need to be further verified by animal models in vivo. However, due to the difficulties in preparation of antigen with native conformational epitopes, induction of functional aab against surface-expressed receptors is a challenging task. We here present a novel strategy by immunizing mice with membrane extracts derived from CHO cells overexpressing human AT1R (hAT1R). The hAT1R-immunized mice developed functional autoantibodies against AT1R which are able to bind to and to activate the native receptor. Furthermore, hAT1R-immunization induced SSc-like disease symptoms including vasculopathy, inflammation and fibrosis in the skin as well as inflammation in the lung. By using a novel immunization strategy, we provide a new animal model for SSc which strongly supports the hypothesis of a pathogenic role of functional aab to AT1R in this disease.
Disease Area Pulmonary Hypertension: Abstract No. 501 – 532
The response of the pulmonary vasculature to hypoxia in isolated lungs of mice expressing the alternative oxidase (AOX) from Ciona intestinalis

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Objective: Hypoxic pulmonary vasoconstriction (HPV) is essential to match local blood perfusion to ventilation and thus to prevent life-threatening hypoxemia under conditions of acute alveolar hypoxia, while chronic hypoxic exposure leads to pulmonary hypertension (PH). Increased production of superoxide originating from mitochondrial complex III has been suggested as underlying oxygen sensing mechanism in both responses. The mitochondrial alternative oxidase (AOX) which is only expressed in lower organisms can bypass complex III and IV when electron transfer at these complexes is inhibited. We thus examined if expression of the AOX of Ciona intestinalis in mice affects HPV.

Results: Isolated lungs from AOXtg mice specifically lacked acute HPV, while the response to the unspecific vasoconstrictive stimulus U46619 was preserved. Inhibition of AOX by n-propyl-gallate (nPG) could restore HPV in lungs from AOXtg mice, while pharmacological inhibition of mitochondrial superoxide release could inhibit HPV in wildtype lungs. Accordingly, isolated PASMC from AOXtg mice showed no hypoxia-induced increase of superoxide which was found in wildtype mice. In contrast, development of chronic hypoxia-induced PH was not different in AOXtg and wildtype mice.

Conclusion: We conclude that mitochondrial superoxide production acts as trigger mechanism in acute, but not chronic hypoxic signaling in the pulmonary vasculature.
Abstract No. 502

**Real life experience with Selexipag at the department of Internal Medicine V, University of Munich**

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Background: Selexipag, an orally available selective prostacyclin receptor agonist, has recently been approved for the treatment of pulmonary arterial hypertension (PAH). The aim of this study was to describe real life experience data of patients with pulmonary hypertension (PH), who were treated with Selexipag.

Methods: We analysed all patients, who were treated with Selexipag from July 2016 to October 2017 in our department. Functional (World Health Organization- functional class (WHO-FC), 6 min walk distance (6 MWD)), serological (pro-BNP) and hemodynamic parameters (pulmonary arterial pressure –mean (PAPm), right arterial pressure-mean (RAPm), cardiac index (CI), pulmonary vascular resistance (PVR)) were collected at baseline and at follow up (FU) after 146 ± 86 days (minimum 82 days, maximum 285 days). Results: 22 patients were treated with Selexipag, Nizza I n=19 (86.4%), Nizza IV n=3 (13.6%). The mean individual maintenance dosage was 2180.0±1023.7µg/d. The baseline PH-medications was endothelin-receptor-antagonist (ERA) + phosphodiesterase-V-inhibitor(PDE-5-inhibitor) n= 17, ERA + soluble guanylatcyclasestimulator (Riociguat) n =3, ERA mono n= 1, ERA + PDE-5-inhibitor + thyrosinkinase-inhibitor (Imatinib) n=1. Currently, follow up data are available in 13/22 (59.1%) patients. Of the remaining 9 patients without FU, 1 died, 1 did not tolerate the lowest dosage of medication, 1 was switched to intravenous treprostinil because of cardiac decompensation before FU. All parameters showed numerical improvement without reaching statistical significance: Δ proBNP -100.8 ± 1096.4 pg/ml (p=0.347), Δ 6MWD+22.5 ± 89.2 m (p=0.346), Δ PAPm -4.4 ± 7.8 mmHg (p=0.064), Δ RAPm -1.4 ± 2.6 mmHg (p=0.109), ΔPVR -1.4 ± 2.9 WE (p=0.133) and Δ CI +0.2 ± 0.6 ml/min/m² (p=0.195). The WHO –FC improved in 4/13 (30.8%) patients and was progressive in 1/13 (7.7%) patient.

Conclusion: Our preliminary data suggest that Selexipag has beneficial effects on functional, serological and hemodynamic parameters in patients with PH in a real life setting.
Abstract No. 503

**Pulmonary veno-occlusive disease: Reevaluation of 58 cases.**

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**Background:** PVOD is a subtype of pulmonary arterial hypertension, associated with an even poorer prognosis and a high mortality rate within few months after diagnosis. It is estimated that PVOD represents 10-15% of cases of idiopathic pulmonary arterial hypertension (IPAH). Since PVOD is poorly understood it remains a challenging clinical differential diagnosis. We therefore retrospectively analysed the course of disease, clinical features, radiologic changes, histomorphologic hallmarks and associated prognosis in 58 cases described in the literature and in 7 cases from Hannover Medical School (MHH).

**Methods:** We studied all available case reports of PVOD in the PubMed database (n=58) in the period between 1934 and 2017 supplemented by an analysis of all PVOD cases which underwent lung transplantation at MHH (n=7) in the period between 2006-2017. Reevaluated parameters were age, sex, symptoms, diagnostics, imaging, histology and treatment.

**Results:** 50% of PVOD patients were lung transplanted or died within a hundred weeks after initial clinical presentation. The interval of first medical presentation and lung transplantation at MHH was closer than in other hospitals. The median age of disease onset was 35.39 years with sex ratio of 1.32 (f): 1 (m). Most patients were definitively diagnosed via autopsy (32.7%) followed by diagnosis by biopsy (29.3%), imaging (22.4%) and explanted lungs (10.3%). Most common histologic change was intima fibrosis of veins and/or venules (90%). However, histologic characteristics were mentioned in only 73.7% of case reports. Radiologic features, which were conclusive in 54% of PVOD cases, were ground glass opacities (77.4%) and septal line thickening (67.7%).

**Conclusion:** One third of the PVOD patients died before being actually diagnosed with PVOD. The diagnostic focus should be placed on lung biopsy, the most sensitive diagnostic procedure, and on computer tomography to distinguish PVOD from other forms of PAH as early as possible.
Abstract No. 504

Vascular cell-specific epigenomic signatures, novel transcription factors and signaling networks in human pulmonary arterial hypertension

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Hypertensive stimuli-induced phenotypic transformation facilitates normal vascular cells to acquire and exhibit pro-proliferative, apoptotic-resistant, pro-inflammatory, pro-fibrotic responses in pulmonary arterial hypertension (PAH). Role of epigenetic alterations in maintenance of these dedifferentiated and mesenchymal stem cell-like phenotypes remains unexplored in PAH, at a genome-wide scale.

We employed next-generation sequencing (NGS) based approaches to profile differential chromatin modification pattern using ChIP-seq, transcriptome profiles using RNA-seq, in ex vivo isolated pulmonary artery adventitial fibroblasts (PAAF) from human donor and idiopathic PAH patients. Therapeutic potential of epigenetic intervention was evaluated ex vivo and in vivo.

Cell-specific transcriptome & methylome profiling revealed differential expression of 2069 genes (2-fold) in PAH-PAAF. To profile alterations in chromatin signatures, we performed ChIP-seq for histone modifications associated with euchromatin (H3K9/14ac, H3K4me3), heterochromatin (H3K27me3, H3K9me3) and enhancers (H3K27ac, H3K4me1). Integrative genomic analysis revealed strong correlation between altered chromatin signatures and transcriptome, which correlated with aberrant protein expression of chromatin modifying enzymes. KEGG analysis of epigenetic signatures revealed association with ABC transporters, cGMP-PKG, NFκB, TGFβ and cancer pathways. Integrative analysis also revealed acquisition of gene expression signatures reminiscent of lung mesenchymal stem cells in PAH, with differential regulation of novel transcription factor networks (ETS, SOX, TBX, ID, GLI, GATA, REL) that may altogether coordinate the pro-proliferative and pro-inflammatory phenotypes in PAH. The aberrant epigenetic signatures in PAH were reversed by targeting chromatin modifying enzymes.

Integrative analysis of vascular cell-specific epigenomics data confirmed that aberrant recruitment of histone modifiers and transcription factors coordinately alter the chromatin landscape and DNA accessibility. These epigenetic alterations consequently mediate an aberrant transcriptional response that facilitates the maintenance of the acquired hypertensive phenotypes that culminates into complex intimal and plexiform lesions in PAH. This genome-scale study also uncovered novel regulatory factors and signaling networks as prospective targets for therapeutic intervention.
Abstract No. 505

HIPPO kinases, MST1/2 regulate vascular remodeling in PAH via targeting FoxO1/3

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Pulmonary vascular remodeling is a key pathological feature of pulmonary arterial hypertension (PAH) characterized by increased proliferation and survival of pulmonary vascular cells including pulmonary arterial adventitial fibroblasts (PAAFs). Recent reports have indicated causative role of YAP/TAZ, co-transcription factors regulated by HIPPO signaling in PAH. However, role of MST1 and MST2 kinases in vascular remodeling are unknown.

In screening studies carried out in lungs and PAAFs from IPAH patients, we observed a strong upregulation of MST1/2 kinases expression in IPAH patients. To study the effect of these kinases on proliferation and apoptosis of PAAFs, overexpression of MST1/2 kinases was carried out in healthy PAAFs. Overexpression of both kinases led to a significant increase in proliferation and survival of the cells, which was reversed by overexpression of catalytic inactive mutants (MST1K59R, MST2K56R) indicating that the effect is mediated via their kinase activity. Furthermore, siRNA mediated knockdown of MST1/2 in IPAH-PAAFs led to strong decrease in proliferation.

Interestingly, MST1/2 kinases were shown in previous reports to phosphorylate and increase nuclear localization of FoxO transcription factors (Lehtinen et al. Cell 2006), recently implicated in pathogenesis of PAH (Savai et al. Nat Med. 2014). As the Hippo pathway and FoxOs share many important functions, including regulation of growth and survival in cells, mechanisms deregulated in PAH, we postulated that MST1/2 might carry out their function via regulation of FoxOs. In contrast to the previous reports, we witnessed decrease in FoxO transcriptional activity with overexpression of MST1/2 kinases in luciferase assay. This effect was reversed on overexpression of kinase inactive mutants. MST1/2 kinases did not effect phosphorylation of FoxO1/3 at Ser/Thr phosphorylated by AKT, indicating effect of MST1/2 kinases on FoxO activity is independent of PI3K/AKT pathway.

Taken together, our data suggests a novel role HIPPO signaling kinases MST1/2 in PAH via regulation of FoxO1/3.
Abstract No. 506

**Safety and efficacy of immunoadsorption as an add-on to medical treatment in patients with severe idiopathic pulmonary arterial hypertension (IPAH)**

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**Background:** Despite optimized medical therapy, severe idiopathic pulmonary arterial hypertension (IPAH) is a devastating disease with poor outcome. Autoantibodies have been detected in IPAH that can contribute to the worsening of the disease.

**Objectives:** The objective of this prospective, open-label, single-arm, multicenter trial was to evaluate safety and efficacy of immunoadsorption (IA) as an add-on to optimized medical treatment in patients with idiopathic pulmonary arterial hypertension (IPAH).

**Methods:** 10 IPAH-patients received IA over 5 days. Clinical parameters including hemodynamics measured by right heart catheter were assessed at baseline and after three and six months. Primary end point was the change in pulmonary vascular resistance (PVR). Secondary endpoints included the change of six-minute walking distance (6MWD), quality of life, safety and plasma levels of IgG and autoantibodies.

**Results:** Evaluation of 10 IPAH-patients (75% female, 51±12 years, 166±10cm, WHO-FC III, 53% on combination therapy) revealed that IA was a safe procedure that efficiently removed IgG and autoantibodies from the circulation. After three months, mean PVR significantly improved by 13.2% (p=0.03), cardiac index improved by 13.1%, no significant changes were found in 6MWD. The quality of life subscale physical functioning significantly improved after 6 months. Serious adverse events in 3 patients were possibly related to IA and included pneumonia, temporary disturbance in attention and thrombocytopenia.

**Conclusions:** IA as add-on to targeted medical treatment in IPAH is a safe procedure with beneficial effects on hemodynamics, especially in patients with high levels of autoantibodies. Larger scaled controlled studies are needed to assess efficacy in IPAH and to identify responders.
Abstract No. 507

Common genetic basis for pulmonary arterial hypertension and high altitude pulmonary edema

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High altitude pulmonary edema (HAPE) and pulmonary arterial hypertension (PAH) are both characterised through an increased systolic pulmonary artery pressure (sPAP). sPAP is also increased in HAPE susceptible subjects at sea level during exercise and during exercise and hypoxia in non-affected family members of PAH patients with PAH gene defects. Therefore, similar physiological mechanisms might be responsible in HAPE and PAH, leading to an abnormal PAP. Both diseases are suspected to have a genetic component contributing to disease manifestation. While several candidate genes are known for PAH genetic analyses of HAPE do not provide a clear picture. Due to the similar phenotype, we hypothesise that mutations of PAH-genes may also be found in HAPE susceptibles.

DNA was extracted from blood of 64 subjects, phenotypically well characterized to be susceptible for HAPE. Mutations were sought in all known PAH genes and further candidate genes using a new PAH-specific gene panel. Next-generation-sequencing was carried out for 42 genes of interest. Any potential pathogenic variants were confirmed with Sanger sequencing and pathogenicity assessed with in silico prediction programmes.

In two HAPE patients mutations in the PAH candidate genes Mothers Against Decapentaplegic Homolog 7 (SMAD7) and Cytochrome P450 Family 1 (CYP1B1) were identified, respectively. The mutations were characterised as pathogenic by prediction programmes and were absent in >120,000 controls. SMAD7 is a pathway gene of the most commonly affected PAH pathway with the bone morphogenic protein receptor 2 (BMPR2) at its centre, and variants in CYP1B1 may act as a modifiers for disease penetrance in BMPR2 mutation carriers.

This is the first study showing possible communalities not only between the physiological determinants of PAH and HAPE but also pointing towards common genetic mechanisms at least in a few patients. Further studies are needed to assess frequency and implication of PAH-gene mutations in HAPE patients.
Abstract No. 508

**Histone Deacetylase 7 mediates hypoxia-induced mitochondrial reprogramming in pulmonary hypertension and cancer**

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Suppression of mitochondrial function promoting proliferation and apoptosis suppression has been described in the pulmonary artery smooth muscle cells from pulmonary arterial hypertension (PAH-PASMCs). Reprogramming of the cellular metabolism towards glycolysis ("Warburg effect"), increased mitochondrial membrane potential (ΔΨₘ) and apoptosis resistance are known hallmarks of cancer as well. Histone deacetylases (HDACs) are enzymes mostly known to work on histones in order to regulated gene transcription. However, recent literature reveal new histone-independent functions, mostly for HDACs class IIa, in the regulation of cellular metabolism. We hypothesized that members of classIIa HDACs might be involved in the cancer-like mitochondrial and metabolic reprogramming of IPAH-PASMCs.

We aim to identify the role and to characterize the mechanism of classIIa HDACs in the regulation of mitochondrial and metabolic remodeling by using hypoxia exposed healthy PASMCs, IPAH-PASMCs and human non-small cell lung cancer cells (A549).

We observe that in PASMCs of human and experimental PH lungs, HDAC7 expression is upregulated. This finding could be reproduced using *ex vivo* exposure of PASMCs to hypertensive stimuli such as hypoxia and growth factors. Pharmacological inhibition and genetic ablation of HDAC7 in IPAH-PASMCs as well as in hypoxia-exposed PASMCs and A549 cells increase glucose oxidation, reduce ΔΨₘ and increase susceptibility to apoptosis through the modulation of pyruvate dehydrogenase kinases (PDKs). Interestingly, modulation of HDAC7 did not affect healthy PASMCs, making the targeting of this molecule selective towards cells with altered phenotype. Moreover, smooth muscle cell specific HDAC7 deletion attenuates PH development in hypoxia-induced mouse model.

Our findings identify HDAC7 as a new player in the modulation of mitochondrial function in PH and cancer. It might represent a link to the several molecular abnormalities so far described and its targeting may affect the diverse signals associated with the two conditions.
Abstract No. 509

End-Stage Chronic Thromboembolic Pulmonary Hypertension: Unique molecular and histopathological imprint

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Background: Chronic thromboembolic pulmonary hypertension (CTEPH) as a multifactorial disease, ultimate to death, if unrecognized and untreated.

Objective: The aim of the project is to identify and compare the transcriptional regulatory landscapes and histological imprints within ongoing and end-stage CTEPH and furthermore with idiopathic PH (IPAH).

Methods: Vascular remodeling quantification and laser capture microdissection of vessels was performed on ex-vivo end-stage CTEPH, IPAH and donor lung tissues, followed by microarray screening. In addition, molecular and histological profiling was carried out on biopsies from ongoing CTEPH. vWF and Ulex europaeus agglutinin I immunoreactivity were accustomed to evaluate the total vessel density. A comparable analysis was implemented on proximal and distal pulmonary endarterectomy (PEA) material.

Results: Morphometric analysis confirmed Gaussian distribution of individual densities of medial hypertrophy measurements, enhanced vascularization and prominent collagen deposition in end-stage CTEPH rather than ongoing CTEPH and IPAH. Bioinformatics analysis of the microarray data revealed differently and commonly regulated genes and gene networks in end-stage CTEPH compared to IPAH. Our in vitro studies, verified that two of the differently regulated genes in CTEPH, CHI3L1 (Chitinase 3-Like-1) and ATX (Autotaxin) promote neovascularization and migration of vascular cells. In distal PEA tissues, elevated insoluble collagen and angiogenic markers were noted as compared to proximal tissues. Importantly, microarray analysis suggests deregulation of several important transcription factor networks in the distal tissue, suggesting their contribution to neovascularization and collagen synthesis.

Conclusion: These studies highlight the histopathological and molecular differences within end-stage and ongoing CTEPH and in comparison to IPAH. Further studies will provide insights into the molecular mechanisms and can possibly contribute to identify novel therapeutic targets.
Abstract No. 510

**Hypoxia alters the redox state and inhibits K+-currents in mouse pulmonary arterial smooth muscle cells**

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Hypoxic pulmonary vasoconstriction (HPV) is a physiological response to localized alveolar hypoxia that is intrinsic to the pulmonary circulation. By hypoxia-induced contraction of pulmonary arterial smooth muscle cells (PASMCs), pulmonary capillary blood flow is redirected to alveolar areas of high oxygen partial pressure (pO₂), thus maintaining the ventilation-perfusion ratio. Although the principle of HPV was recognized decades ago the underlying pathway still remains elusive.

Voltage gated K+-channels (Kv-channels) are known to be redox-sensitive and crucial for mediating membrane potential in PASMCs - thereby controlling Ca²⁺-entry and subsequently vascular tone. Here, we investigated whether acute hypoxia alters the redox state of isolated PASMCs from mice and if this impacts Kv-channel activity, membrane potential and intracellular Ca²⁺- level.

Kv-currents and membrane potential in response to hypoxia were investigated by whole cell patch clamp experiments (voltage and current clamp, resp.) on freshly isolated murine PASMCs. Simultaneously, pO₂ was recorded using an optical oxygen sensor and intracellular Ca²⁺ was measured by ratiometric FURA-2 imaging. The redox state was monitored by Raman spectroscopy using an excitation wavelength that was in resonance with the hemeproteins (532 nm).

Raman-spectra values for reduced myoglobin and cytochrome c both increased upon exposure of PASMCs to hypoxia (4% O₂) and returned to the initial value upon reverting to normoxia (21 % O₂), thus indicating a shift in the cells’ redox state. In accordance, Kv-currents were dose-dependently and reversibly inhibited by acute hypoxia, resulting in a depolarization of PASMCs, thus triggering a rise in intracellular Ca²⁺.

In conclusion, hypoxia induced a shift in redox state in PASMCs, thereby mediating the inhibition of Kv-channels. The resulting depolarization and Ca²⁺-entry may account for the contraction of PASMCs – thus initiating HPV.
Abstract No. 511

Reduced right ventricular reserve and impaired Pulmonary Arterial Compliance in patients with Systemic Sclerosis and concomitant borderline pulmonary arterial pressures

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Background and aims: Borderline pulmonary arterial pressures (BoPAP) in patients with systemic sclerosis (SSc) are a frequent finding and could represent an intermediate stage between normal pulmonary pressures and manifest pulmonary hypertension (PH). The aim of this study was to characterize the difference in right ventricular response to exercise between normal hemodynamics and BoPAP-patients.

Methods: Patients with confirmed SSc (n=112) underwent right heart catheterization (RHC) at rest and during exercise and were divided into three groups according to their resting mPAP values: normal mean PAP (mPAP ≤ 20 mmHg), BoPAP (mPAP 21-24 mmHg) and manifest PH (mPAP ≥ 25 mmHg).

Results: SSc patients with BoPAP showed significantly lower right ventricular contractile reserve than SSc patients with normal hemodynamics as shown by reduced cardiac output (CO) and cardiac index (CI) increase during exercise measured by RHC (Δ CO 3.51 ± 2.50 vs. 5.76 ± 2.56 l/min., p< 0.0001; Δ CI: 3.31± 1.52 vs. 2.11± 1.18 l/min/m², p< 0.0001).Patients with BoPAP had a significantly lower pulmonary arterial compliance (PAC) than patients with normal mPAP at rest (p=0.016), 25 (p=0.033) and 75 (p=0.024) Watts.

Conclusion: SSc patients with BoPAP already display a reduced RV reserve together with impaired PAC, when compared with SSc patients and normal resting hemodynamics. This is additional evidence for BoPAP being an early stage of pulmonary vascular disease.
Abstract No. 512

**The so-called ligamentum arteriosum is an innervated muscle**

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**Introduction**: The ligamentum arteriosum is generally considered to be a mere a remnant of the embryonic bypass (ductus arteriosus) from the pulmonary circulation to the aortic arch, obliterating soon after childbirth. Based on initial observations of a high density of nerve fiber in this region, we set out to elucidate the morphology and innervation of the ligamentum arteriosum. We used appropriate mouse reporter strains to highlight cholinergic (choline acetyltransferase* = ChAT*) and autonomic postganglionic nerve fibers (nicotinic receptor subunit alpha3* = chrna3*).

**Method**: The mediastinum of reporter mouse strains (*chrna3*-eGFP, n=8; *chat*-eGFP, n=4) and 4 human ligaments obtained from body donors were subjected to single- and double-labeling immunofluorescence using antibodies against α-smooth muscle actin (aSMA), protein gene product 9.5 (PGP9.5), tyrosine hydroxylase (TH, noradrenaline synthesizing enzyme), neuropeptide Y (NPY), calcitonin gene-related peptide (CGRP), and neurofilament 68 (Nf68). Endogenous eGFP-fluorescence was enhanced by eGFP-immunolabeling.

**Results**: In mice, the lumen of the “ligament” is not completely obliterated, luminal remnants were also observed in human. Contrary to a canonical ligament, the ligamentum arteriosum is mainly made up by longitudinally arranged aSMA-positive cells. The point of attachment of the ligament to both the aortic arch and pulmonary trunk received a noticeable amount of *chrna3*-eGFP, TH-positive and NPY-positive nerve fibers in mice, but no cholinergic innervation. This innervation is much denser than that of the aortic arch and pulmonary trunk. In human ligaments, PGP9.5-, Nf68- and CGRP-positive fibers were observed next to smooth muscle cells (aSMA-positive).

**Conclusion**: The so-called ligamentum arteriosum is a muscular structure with noticeable, presumably sympathetic and to a lesser extent sensory innervation, at its points of attachment to the pulmonary trunk and aortic arch. It might influence impedance/compliance of the vascular segments to which it is attached, which are the best prognostic parameters for patient survival in pulmonary hypertension.
Abstract No. 513

Role of Peptidylprolyl cis/trans Isomerase, NIMA interacting 1 (PIN1) in Pulmonary Hypertension

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Rationale: Pulmonary arterial hypertension (PAH) is a progressive disease of various origins that is associated with a poor prognosis and results in right heart dysfunction. An upsurge of vascular cell proliferation and their resistance to apoptosis are prominent features of pulmonary vascular remodeling in PAH. Proline (Pro)-directed Ser/Thr phosphorylation (pSer/Thr-Pro) is a common signaling mechanism in cells. PIN1 (Peptidylprolyl cis/trans isomerase, NIMA interacting 1) catalyzes the isomerization of prolines between cis- and trans-, acting as a molecular switch in coordinated cellular processes. The expression of PIN1 and its impact on the emergence of Pulmonary Hypertension (PH) has not been investigated. Our aim is to identify the role of PIN1 in PH.

Methods: Proliferation of human pulmonary artery smooth muscle cells (hPASMCs) was assayed by monitoring of BrdU incorporation. TUNEL assay was employed for the detection of apoptosis. The PIN1 knock-down (siRNA targeting approach) was followed by proliferation assays and Western Blot analyses of proliferative and apoptotic markers. Immunofluorescence stainings were performed in human cryopreserved lung tissues.

Results: Upon investigation, the expression of PIN1 was found to be up-regulated in diseased lungs of patients with idiopathic PAH (IPAH), particularly in the smooth muscle layer, as well as in human IPAH PASMCs. It was observed that the levels of PIN1 escalated upon hypoxia stimulus in mouse SMCs. Importantly, small interfering RNA based knockdown of PIN1 in hPASMCs diminished the proliferation of cells induced by Platelet-derived abbreviation (PDGF-BB), as determined by BrdU assays and Western Blot analyses of proliferative markers. PIN1 inhibitor, Juglone inhibited the proliferation and induced apoptosis in hPASMCs in a dose-dependent manner. Silencing of PIN1 restricted the accumulation of HIF1-α upon hypoxia stimulus.

Conclusion: These results indicate that PIN1 might act as a modulator of cell cycle progression resulting in an increase in vascular cell proliferation and resistance to apoptosis.
Abstract No. 514

Supportive therapy within the modern treatment era of idiopathic pulmonary hypertension: Impact of long term oxygen supply

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Background: In patients with idiopathic pulmonary arterial hypertension (iPAH) current guidelines recommend a long-term oxygen supply. However, the prognostic impact in iPAH patients is unknown.

Methods: Retrospective we analyzed iPAH patients entered into the Giessen Pulmonary Hypertension Registry between January 2000 and April 2017 with either documented absence of oxygen therapy or documented use until end of follow up. The primary outcome was 5 years’ overall survival obtained from the registry, end of follow up was July 2017. The prognostic relevance of oxygen therapy was assessed by Kaplan–Meier analyses stratified for disease severity.

Results: 198 iPAH patients were included (median PVR 878 [IQR 1265 – 592] dyn; cardiac output 3.9 [IQR 4.7-3.1] l/min, pO2 69 [IQR 61-77] mmHg, DLCO 60 [IQR 72-46]%pred., age 59 [IQR 72-46] years) of which 119 were receiving long-term oxygen therapy. Patients receiving no oxygen supply showed a superior overall 5 year survival when stratified by preserved cardiac output (91.1%) or lower PVR (85.2%). A subgroup of patients with preserved hemodynamics and oxygen therapy showed an overall 5 year survival almost comparable to patients with impaired pulmonary hemodynamics (with and without oxygen therapy) (stratified by cardiac output (48.6 %vs66.4%vs61.3%); stratified by PVR (54.4%vs70.0%vs51.6%) (overall log-rank \( p < 0.001 \)). This subgroup with preserved hemodynamics and oxygen therapy was presenting with a severely impaired DLCO and lowest pO2 in comparison (\( p< 0.05 \) across all groups).

Conclusions: Not surprisingly patients without oxygen therapy and preserved pulmonary hemodynamics showed the best overall survival. A subgroup of patients with preserved pulmonary hemodynamics and oxygen therapy showed an impaired overall survival almost comparable to patients with advanced disease. Our data indicate that these high risk patients might resemble the proposed "pulmonary-phenotype" with reduced DLCO. Our preliminary data indicate that oxygen therapy might not be able to influence the clinical course of patients with advanced disease or the "pulmonary-phenotype" of iPAH.
Abstract No. 515

The role of mitophagy in development of pulmonary hypertension

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Background: Pulmonary hypertension (PH) is characterized by pulmonary vascular remodeling leading to increased pulmonary vascular resistance and ultimately right heart failure. Mitochondrial dysfunction plays an important role in the development of PH. Mitophagy serves to remove dysfunctional mitochondria mostly via phosphatase and tensin homolog (PTEN)-induced putative kinase 1 (PINK1). We aimed to investigate the role of mitophagy in hypoxia-induced PH.

Methods: PH was induced in wild type (WT) and in global PINK1 knockout (PINK1−/−) mice exposed for 4 weeks to chronic hypoxia (10% O2 in the ambient air) and quantified by hemodynamics, echocardiography and morphometry. Proliferation and apoptosis of primary pulmonary artery smooth muscle cells (PASMCs) were studied after incubation at 1% O2 for 5 days.

Results: Protein expression of PINK1 was increased in lung homogenate from mice with chronic hypoxia-induced PH as well as in PASMC exposed for 5 days to 1% O2. Hypoxia-induced proliferation of PINK1−/− PASMC was decreased while their apoptosis was increased after incubation in hypoxia compared to WT PASMC. Accordingly, the degree of hypoxia-induced pulmonary vascular muscularization after chronic hypoxic exposure of mice (4 weeks, 10% O2) was lower in PINK1−/− compared to WT mice. However, the increase of right ventricular systolic pressure, degree of right heart hypertrophy and heart function was not significantly different in the mice of different genotype and gender.

Conclusion: Our data suggests that PINK1-mediated mitophagy affects pulmonary vascular remodelling in chronic hypoxia-induced PH. However, detailed cell-type specific mechanisms and in vivo effects on pulmonary vascular resistance have to be further investigated.
Abstract No. 516

The role of Brain derived neurotrophic factor in chronic hypoxia-induced pulmonary hypertension

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Objective: In vitro stimulation of murine pulmonary arterial smooth muscle cells (PASMC) with Brain derived neurotrophic factor (BDNF) results in increased proliferation. Moreover, mRNA expression of BDNF in mice and human PASMCs after chronic hypoxic exposure is increased. However, the role of BDNF in chronic hypoxia-induced pulmonary hypertension (PH) is still unknown. Therefore, we investigated the effect of BDNF deletion in smooth muscle cells (SMC) in mice on the development of chronic hypoxia-induced PH.

Results: In vitro experiments showed that exposure of PASMC to hypoxia (1%O2) for five days increased the release of BDNF into the medium. Right ventricular systolic pressure determined by in vivo hemodynamic was not different in SMC specific BDNF knockout mice (BDNF−/−) and respective control mice after exposure to chronic hypoxia (4 weeks, 10% O2). Moreover, the degree of muscularization of small pulmonary arteries after chronic hypoxia did not differ between the genotypes. However, BDNF−/− mice had a significantly lower decrease of both right ventricular internal diameter and tricuspid annular plane systolic excursion after chronic hypoxic exposure measured by echocardiography.

Conclusion: SMC specific knockout does not affect development of chronic hypoxia-induced PH in vivo. The specific effect on right ventricular function has to be further investigated.
Abstract No. 517

The physiological significance of P2Y2 receptor in the development and treatment of PAH

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Objective: Elevated blood pressure in pulmonary arterial hypertension (PAH) is a result of pathological processes that involve G protein-coupled receptors (GPCRs). Crucial mediators of vasodilation, such as nitric oxide (NO), are released from endothelial cells in response to elevated blood pressure. Recent studies have demonstrated the participation of purinergic receptor P2Y2, a GPCR, in NO secretion in response to fluid shear stress that results in systemic vasodilation. The aim of our investigations is to examine a possible involvement of P2Y2 in the development and treatment of PAH.

Results: P2Y2 is significantly downregulated in the pulmonary vasculature of PAH patients compared to non-PAH (healthy) donors at mRNA level whereas the specific P2Y2 protein was upregulated. The mRNA downregulation can be reproduced in human pulmonary arterial endothelial cells (HPAECs) when exposed to hypoxic conditions (1% O₂) for 72 hours. The stimulation of HPAECs with MRS2768, an agonist of P2Y2, stimulates the phosphorylation of Src, Akt, Erk1/2, and eNOS in time- and concentration-dependent manners to increased secretion of NO. The increased phosphorylation of the later cascades is associated with elevated concentrations of inositol 1, 4, 5-triphosphate (IP3) which refers to a specific GPCR(s)-involvement in the signaling events. On the other hand, inhibition of P2Y2 decreased the activations observed so far. This agonist shows no significant increased proliferation rate or cytotoxicity in HPAECs.

Conclusion: In this study, we demonstrate that P2Y2/MRS2768 interactions lead to significant stimulation (phosphorylation) of Src, Erk1/2, Akt and eNOS, a signaling cascade leading to increased endothelial NO secretion under normoxic conditions in vitro. The increased concentrations of IP3 point towards the involvement of Gq/11 proteins interacting with P2Y2. More investigations and implementation of perfused lung together with in vivo approaches might help to unveil the physiological function of P2Y2 signaling in the pulmonary arteries.
Abstract No. 518

**Case report: Treatment with low-dose tacrolimus attenuates bleeding complications but not pulmonary hypertension in a patient with hereditary hemorrhagic telangiectasia**

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Hereditary hemorrhagic telangiectasia (HHT) is an autosomal dominant disease characterized by arteriovenous shunts that manifest in the microvasculature as mucocutaneous telangiectases and visceral vascular malformations. Severe and chronic bleeding of the telangiectases is a common and life threatening complication that often requires blood transfusion and limits quality of life. Moreover, mutations that lead to HHT, such as of the activin A receptor-like type 1 (ACVRL1 or ALK-1) gene, which encodes for a bone morphogenic protein type I receptor of the transforming growth factor-β family of ligands, also predisposes for pulmonary hypertension (PH). Here we report of a patient with an ALK-1 mutation with severe PH and bleeding complications due to HHT. The patient was treated with an oral combination of specific PH therapy and started treatment with tacrolimus that was previously shown to increase ALK-1 expression and improve severe PH in case reports. Furthermore, tacrolimus can inhibit release of vascular endothelial growth factor (VEGF) which is important in disease progression of HHT. Treatment with low-dose tacrolimus could lead to a dramatic improvement of the nose bleeds (epistaxis) in that patient, however course of PH was not influenced. In parallel, increased VEGF plasma levels, as well as VEGF expression in peripheral blood mononuclear cells was decreased by tacrolimus treatment to the level of control patients. In addition, TGFβ ligands expression and secretion which was attenuated at basal level is increased by tacrolimus treatment.
Abstract No. 519

Mutations in the bone morphogenic protein receptor 2 promoter in heritable pulmonary arterial hypertension

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Background: Mutations have been identified in the bone morphogenetic protein receptor II (BMPR2) gene as a predominant cause for pulmonary arterial hypertension (PAH) in about 85% heritable PAH (HPAH) and 25% of idiopathic PAH (IPAH) cases. However, the penetrance of BMPR2 mutations is only about 27% indicating that other modifiers such as promoter variants may contribute to disease manifestation. Mutations of the BMPR2 promoter have been identified previously as a “second hit” in a family with autosomal dominantly inherited PAH.

Objectives: The aim of this study was to identify BMPR2 promoter mutations in HPAH and sporadic cases patients and to analyse their transcriptional effect on the BMPR2 gene expression in human pulmonary artery smooth muscle cells (hPASMCs).

Methods: BMPR2 promoter variants were screened by direct Sanger sequencing and next generation sequencing in IPAH/HPAH patients and their effect was investigated on BMPR2 promoter expression in vitro. The BMPR2 promoter regions (1520 bp) containing identified variants were amplified together with the wild-type promoter and cloned into the pGL4.10 vector fused with a luciferase reporter gene. Recombinant plasmids were transfected into hPASMCs and transcriptional activity was assessed.

Results: Nine different BMPR2 promoter variants were identified in PAH families and IPAH patients by Sanger sequencing. In the functional analysis, seven of the nine variants (c.-575A>T, c.-586dupT, c.-910C>T, c.-1141C>T, c.-930_-928dupGGC, c.-933_-928dupGGCGGC and c.-930_-928delGGC) led to a significantly decreased transcriptional activity in comparison to the wild-type. In silico prediction programmes revealed that these seven variants may have an impact on BMPR2 function in patients.

Conclusion: This study identified new mutations in the BMPR2 promoter region which might functionally affect the BMPR2 gene expression. The mutations might act as a “second hit” at least in some families as they reduce the gene expression.
Abstract No. 520

5,6-epoxyeicosatrienoic acid induces both dilation and constriction in mouse intrapulmonary arteries

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Epoxyeicosatrienoic acids (EETs) are known to be potent vasodilators in systemic circulation. However, effects of EETs in pulmonary circulation are not completely clear. The aim of this study was to investigate the effects of EETs on tone in mouse intrapulmonary arteries (IPA).

The contractile responses were measured in isolated mouse IPA (80–200 µm in diameter) using wire myography. All four EET regioisomers (5,6-,- 8,9-, 11,12- and 14,15-EET) had no effect on tone in IPAs in the resting state at the concentration range of 0.01–1.50 µM. However, after a preconstriction with a thromboxane A2 mimetic U46619, IPAs responded to all EETs (1.5 µM) with a significant sustained relaxation, with the response to 5,6-EET being less pronounced compared to other regioisomer. By contrast, in IPAs preconstricted with depolarization-inducing agent, KCl, 5,6-EET evoked a biphasic constriction in IPAs consisting of an initial transient response with a peak at 3–4 min, followed by a sustained increase in IPA tone. Other EETs, as well as metabolites of 5,6-EET (5,6-dihydroxyeicosatrienoic acid and 5,6-EET-ethanolamide), had no effect in the presence of the KCl-induced preconstriction. All responses to 5,6-EET were abolished by the EET receptor blocker 14,15-EEZE (1.5 µM). 5,6-EET induced significant inward whole-cell currents in depolarized pulmonary artery smooth muscle cells at a holding potential of -20 mV, but not at -40 mV, which is close to resting membrane potential. The observed currents may result from Ca²⁺ entry, since 5,6-EET caused a rise in intracellular Ca²⁺ levels in the presence of KCl-induced preconstriction, as measured by Cal-520 fluorescence.

While all EET regioisomers relax mouse IPAs, 5,6-EET is also able to induce biphasic vasoconstriction. The resulting response to 5,6-EET appears to be dependent on the membrane potential of pulmonary artery smooth muscle cells. 5,6-EET may play an important role in the regulation of vascular tone in pulmonary circulation.
Replacement of ESP by mPAP to calculate right-ventricular –arterial coupling leads to underestimation of ESP and overestimation of Ees/Ea

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Background: Pulmonary arterial hypertension (PAH) is associated with high morbidity and mortality. Right ventricular function is of paramount prognostic importance in PAH, and there is increasing interest in the assessment of ventriculo-arterial coupling. In high-ranked publications end systolic pressure (ESP) was replaced by mean pulmonary arterial pressure (mPAP) to simplify the most frequently used single-beat-estimation of ventricular elastance (Ees)/arterial elastance (Ea) in the right ventricle. The aim of the study was to analyze the relationship between mPAP and ESP in PAH-patients

Methods: In a prospective study we included 20 consecutive patients with PAH. Conductance catheter (4F, CD Leycom, Hengelo, The Netherlands), CD Leycom, Hengelo, The Netherlands) and Right heart catheter were done at the same day, cardiac MRI to calibrate the volume-measurements of the conductance-catheter was driven one day before.

Results:

In 20 consecutive patients with PAH who gave their informed consent (13 females; median [interquartile range] pulmonary vascular resistance: 463.5 [348.0] dyn·s/cm²; mean ± standard deviation mixed venous oxygen saturation: 69.0 ± 6.1%; mean pulmonary arterial wedge pressure: 7.1 ± 2.4 mm Hg) we observed a strong correlation between mPAP and right ventricular ESP in our PAH cohort, consistent with previous results in patients without PAH. The association was described by a linear regression model as ESP = 1.65 × mPAP – 7.79 (r = 0.932 r² = 0.868; p < 0.001). Bland–Altman analysis of our PAH cohort revealed that the mPAP values underestimated the absolute ESP values throughout the observed pressure range, with absolute deviations increasing with the magnitude of ESP.

Conclusions:

In one of the most frequently applied single-beat-methods to calculate right ventriculo-arterial coupling, Ees is calculated as (Pmax – ESP)/stroke volume (SV). Replacing ESP by mPAP leads to overestimation of Ees and underestimation of Ea. Both deviations lead to overestimation of ventriculo-arterial coupling (Ees/Ea).
Abstract No. 522

**Significant association of pressure-volume-loops derived right ventriculo-arterial coupling with right and leftventricular MRI-strain**

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**Background:** Pulmonary arterial hypertension (PAH) is associated with high morbidity and mortality. Cardiac Magnetic resonance tomography (MRI) has gained importance in diagnostic of right ventricular function. MRI-derived-Strain-analysis of the right ventricle is a promising tool for assessing right ventricular function. However, strain-analysis of the right ventricle has not been compared to ventriculo-arterial coupling yet.

**Methods:**

28 consecutive PAH-patients were imaged by 1.5T-MRI (Siemens Healthcare, Germany) and analysed for circumferential, radial and longitudinal strain with an analysing software (Circle cardiovascular imaging Inc, Canada). Conductance catheter (4F, CD Leycom, Hengelo, The Netherlands) and Right heart catheter were done the day after MRI. Ventricular-arterial-coupling measurements were based on measurements of pressure-volume-loops of the right ventricle using a single-beat-estimation of Ventricular elastance (Ees) (Pmax-ESP/SV).

**Results:** In 28 patients (age: 56±13 years) with PAH (mean pulmonary arterial pressure: 40±13 mmHg, pulmonary vascular resistance: median 498 [IQR 688-338] dyn) the relationship between rightventricular Ees (median 0,48 [IQR 0,78-0,35] and arterial elastance (Ea) (median 0,74 [IQR 1.0-0.44] Ees/Ea (median 0,73 ±0,44) was compared with MRI-derived strain. We observed significant correlations between MRI- RV global longitudinal strain (Rho -0.5, p: 0.007), RV global radial strain (Rho: 0.46, p: 0.014), LV radial strain (Rho:0.59, p: 0.001) and LV circumferential strain (-0.575, p: 0.001).

**Conclusions:**

Right ventricular-arterial coupling significantly correlates with right and left ventricular strain. The correlation with left ventricular strain indicates an improvement of left ventricular function in higher right ventricular Ees/Ea relationships and highlights the importance of ventricular interdependency.
Abstract No. 523

Wnt-Signaling Pathway drives right ventricular remodeling

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Wnt signaling pathway controls heart development but is also modulated during adult heart remodeling. Although much is known about mechanisms triggering left ventricular hypertrophy, little is known about signaling cascades leading to Right ventricle (RV) hypertrophy and failure. Since Wnt signaling was shown to be important for the development of RV, which is the major determinant of functional state and prognosis in pulmonary hypertension (PH). We hypothesize that it may play a role in RV fibrosis and RV failure.

Methods and Results: In this study, we employed pulmonary artery banding (PAB) and monocrotaline (MCT) animal models to induce RV hypertrophy and RV failure in rats and mice. Cardiac MRI and invasive hemodynamic measurements were performed after 3 weeks in PAB- and sham-operated mice/rats respectively. The PAB and sham mice were randomized and treated for 2 weeks with LGK974 (Porcupine inhibitor) or vehicle treatment. Subsequently, the RV were investigated for fibrosis assessment and analyzed for gene expression.

We found significant upregulation of several Wnt signaling molecules: GSK3β, β-catenin, Frizzled1 in the RV and fibroblasts isolated from RV of PAB and MCT rats. Immunohistochemical analysis of hypertrophic human hearts also showed an increased β-catenin expression compared to donor hearts. Genetic inhibition of β-catenin, in vitro significantly regulated collagen synthesis and fibroblast proliferation. Importantly, MRI data revealed a significant increase in the RV mass, end diastolic volume, end systolic volume and decrease in ejection fraction of RV of PAB mice, upon LGK treatment they could recover significantly. mRNA expression of β-myosin heavy chain, natriuretic peptides, collagen family members were increased in PAB which were significantly decreased in LGK treated PAB mice.

Conclusion: Therapeutic targeting of WNT receptors and its downstream signaling molecules could offer potential treatment strategies for RV fibrosis and failure.
Abstract No. 524

Generation of disease-specific BMPR2-mutated iPSCs and development of transgenic reporter cell lines as tools for pulmonary hypertension disease modelling and drug discovery

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Introduction: Pulmonary hypertension (PH) is a progressive disease in which pre-capillary pulmonary arteries are obstructed concomitant with increased arterial pressure leading to right heart failure. Mutations in bone morphogenetic protein receptor 2 (BMPR2) resulting in reduced transcription of target genes linked to proliferation and apoptosis in endothelial cells (ECs) and smooth muscle cells occur frequently in PH. Despite molecular insights mainly from studies with primary cells or immortalized cell lines, knowledge about the underlying mechanisms of PH is limited. Induced pluripotent stem cells (iPSCs) can proliferate and differentiate into cell types of all three germ layers in vitro. Therefore, iPSCs offer an attractive cell source for the generation of functional human ECs from PH patients and healthy individuals. This project aims at generating ECs from PH patient-specific iPSCs harbouring BMPR2 mutations as an in vitro disease model to elucidate the role of EC malfunction in PH and for high throughput drug screening.

Methods: PH patient-specific iPSCs were generated from CD34pos cells using sendai virus reprogramming vectors. BMPR2mutated iPSCs could be differentiated to ECs by timed application of growth factors (BMP4/VEGFA) and activation of the WNT pathway. Applying a reporter assay for Smad4-dependent transcription with luciferase readout will allow for studying impaired BMP-target gene transcription in ECs derived from BMPR2mutated in comparison to BMPR2wildtype iPSCs. Results: PH-patient specific iPSCs demonstrated expression of pluripotency associated markers on both mRNA and protein levels. Differentiated BMPR2mutated ECs showed expression of typical EC markers (VE-Cadherin, CD31) and formed networks in matrigel assays. Furthermore, when cultured on fibronectin coated plates, generated ECs proliferated and doubled their population over several passages while maintaining CD31 expression.

Conclusions: Using BMPR2mutated ECs derived from PH patient-specific iPSCs could be a valuable tool for heritable PH disease modelling and drug discovery for PH therapy.
Inhibition of ADORA1 and PDE10A as a combination therapy for pulmonary arterial hypertension

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Despite substantial advancements in the treatment of pulmonary arterial hypertension (PAH), obstacles still remain in achieving the optimal outcomes. Various treatments have been developed to target signaling pathways which leads to increased intracellular cAMP. Adenosine signaling pathway is well known to regulate intracellular cAMP in strict manner depending on the adenosine concentration and adenosine receptors availability. However, the role of adenosine signaling in PAH pathogenesis remain largely unexplored.

From the available RNA-seq data, it was observed that Adenosine A1 Receptor (ADORA1) was highly expressed under the disease condition. Alternatively, inhibition of cAMP hydrolyzing phosphodiesterases (cAMP-PDEs) to increase cAMP levels demonstrated beneficial effects in the experimental models of PAH. Screening for cAMP-PDEs colocalised with ADORA1, it was observed PDE10A was in close proximity to ADORA1 compared to other PDEs. Co-immunoprecipitation experiments confirmed ADORA1 and PDE10A were interacting with AKAP5 under diseased condition. PDE10A was also upregulated under the disease condition. From the in vitro studies it was observed that dual (genetically and pharmacological) inhibition of ADORA1 and PDE10A induced pro-apoptotic and anti-proliferative effect in human pulmonary artery smooth muscle cells (hPASMCs) via increased cAMP levels. Impressively, in in vivo studies, the dual inhibitor treated MCT-PAH rats have shown better survival rate compared to the placebo. The hemodynamics and MRI data have shown that dual inhibitor treated MCT-PAH rats were having lower pulmonary vascular resistance and reduced right ventricular hypertrophy. The morphometric analysis revealed reduced medial wall thickness and muscularization with dual inhibition treatment. It is very well known that tightly regulated cAMP microenvironment exist within the cell. It was observed that dual inhibition of ADORA1 and PDE10A showed a molecular signature similar to that of increased cAMP/PKA signaling. These results show that this combination is a promising step towards having a better treatment in the field of pulmonary hypertension.
Role of SPARC in dysregulated pulmonary arterial smooth muscle cell function in pulmonary hypertension

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IntroductionPulmonary hypertension (PH) is a severe disease which can cause mortality. It is characterized by excessive pulmonary vascular remodeling, leading to elevated pulmonary arterial pressure and right heart hypertrophy. PH is caused among others by chronic hypoxia, vasoconstrictor/vasodilator and/or growth factor imbalance, leading to pulmonary arterial smooth muscle cell (PASMC) dysregulation. Chronic hypoxia-induced PH in mice can be reversed by re-exposure to normoxia. AimUntil now, research about PH concentrates mostly on the onset and development of PH. In this study, we focus on the mechanisms underlying the reversal of PH. By using the microarray technique, we aim to identify potential candidate genes contributing to reverse remodeling, specifically in the pulmonary vasculature.

MethodsReverse remodeling was investigated in adult mice (C57BL/6J) either exposed to normoxia (21% O2), chronic hypoxia (10% O2), or chronic hypoxia with subsequent re-exposure to normoxia for 1, 3, 7, 14 days. Pulmonary vessels were laser-microdissected followed by RNA isolation and microarray analysis. Regulation of potential candidate genes was confirmed by quantitative real-time PCR. In addition, the functional effect was assessed in vitro in human primary PASMC and in vivo in respective knockout mice. ResultsIn laser-microdissected murine pulmonary vessels, we identified secreted protein acidic and rich in cysteine (SPARC) as one gene down-regulated in all re-oxygenation time points investigated. Hypoxia-dependent SPARC regulation was confirmed in primary human PASMC and in chronic hypoxic mice. SPARC knock-down in PASMC led to decreased proliferation and Akt activation. Molecular analysis showed HIF-2α-dependent SPARC expression following hypoxic stimulation. In addition, SPARC was elevated expressed in human idiopathic pulmonary arterial hypertension patients. Moreover, role of SPARC was assessed in vivo in SPARC-/- mice. ConclusionsIn conclusion, we suggest SPARC as a novel potential contributor for pulmonary vascular remodeling. This is however the first report revealing the association of SPARC with PH.
Abstract No. 527

Effects of the multikinase inhibitor Regorafenib on pulmonary arterial smooth muscle cell function

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Rationale: Pulmonary arterial hypertension (PAH) is characterized by mean arterial pressure >25 mmHg with increase in pulmonary vascular resistance followed by right ventricular failure and death. Inhibition of receptor tyrosine kinases has shown a beneficial role in treatment of pulmonary hypertension. Regorafenib (BAY 73-4506) is a kinase inhibitor that targets VEGFR 1-3, RET,C-KIT, PDGFR, FGFR, RAF-1 and p38 MAP Kinases. Regorafenib has been clinically approved for the treatment of both metastatic colorectal cancer and gastrointestinal stromal tumours in Imatinib and Sunitinib non-responders. As p38 MAPK inhibition is known to reverse PAH phenotype and Regorafenib has demonstrated p38 MAPK inhibitory effects in numerous studies, we thought to investigate the potential of Regorafenib as a therapeutic strategy for the treatment of PAH.

Methods: The proliferation of human pulmonary arterial smooth muscle cells (hPASMCs) from controls (donor) and patients with idiopathic PAH (IPAH) was induced by Platelet derived growth factor (PDGF-BB). Human PASMCs proliferation was determined by 5-bromo-2'-deoxyuridine (BrdU) incorporation assay. Cell migration was assessed by Transwell Chamber assay. The rate of hPASMCs apoptosis was examined by Terminal deoxynucleotidyl transferase (TdT) dUTP Nick-End Labeling (TUNEL) assay.

Results: Treatment with Regorafenib has shown a strong anti-proliferative effect on PDGF-BB induced proliferation in both donor and IPAH hPASMCs. Regorafenib inhibited PDGF BB induced migration of hPASMCs in a dose dependent manner. Similarly, Regorafenib demonstrated remarkable anti-migratory effects in hPASMCs. TUNEL assays revealed that Regorafenib does not induce apoptosis of smooth muscle cells.

Conclusion: Our in vitro data suggests that Regorafenib demonstrates anti-proliferative and anti-migratory effects which are crucial for the treatment of PAH.

Future Prospects: Based on our in vitro data we aim to investigate the effect of Regorafenib in experimental models of PAH.
Abstract No. 528

Cell-cycle inhibition for the therapy for pulmonary arterial hypertension

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The remodeling of small pulmonary arteries represents a main pathological finding associated with pulmonary arterial hypertension (PAH). Hyper-proliferation of pulmonary vascular cells results in the obstruction of the vessels leading to increased pulmonary artery pressure. These vascular lesions of patients with severe PAH exhibit a tumor-like phenotype e.g. an uncontrolled replicative potential. We hypothesize that an increased activity of cyclin-dependent-kinases (CDK) is responsible for the unlimited cell growth of the pulmonary vascular cells.

We could identify several CDKs to be strongly over-activated in IPAH patient derived PASMCs. Next, we confirmed also an up-regulation of CDK expression in human specimen but also in animal models of P(A)H. We tested pharmacological CDK inhibitors (CDK-Is) to block pulmonary artery smooth muscle cell (PASMC) proliferation in vitro and in vivo. These experiments served as a proof-of-principle study and allowed us to determine the efficacies of different CDK-Is. CDK-Is clearly reduced the proliferation of PASMCs in XTT and BrdU assays. Flow cytometric measurements indicated an arrest in cell-cycle progression after incubation with CDK-Is. Cell death detection assays using AnnexinV/PI as well as LDH could rule out the induction of apoptosis. No signs of cytotoxicity of the compounds have been observed. Furthermore, we could show a diminished phosphorylation of the direct CDK downstream target and proliferation marker P-Rb (Retinoblastoma protein) by Western blot analysis in PASMCs which were treated with CDKIs.

In vivo, CDK-Is showed a significant improvement of several parameters of right ventricular function/hypertrophy and hemodynamics, especially in the monocrotaline rat model. The analysis of the pulmonary tissue ex vivo showed a targeted effect of CDK-Is as determined by the decrease of the percentage of fully muscularized pulmonary vessels and impaired downstream CDK signaling. We can conclude that there is rationale in investigating the therapeutic potential of CDK-Is as a new treatment option for PAH.
Abstract No. 529

JAK/STAT Signaling in Pulmonary Arterial hypertension

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Background:

Lung vascular remodeling is the hallmark of pulmonary arterial hypertension (PAH) and attributed to the increase of proliferation and resistance to apoptosis of pulmonary vascular cells. A number of proliferative mediators including platelet-derived growth factor (PDGF) and cytokines are thought to play an important role in the pathogenesis of the abnormal phenotype of the pulmonary vascular cell in PAH, through activating the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway.

An effective strategy using potent small molecule inhibitors should, therefore, target those ligand-induced receptor tyrosine kinases (RTKs), and their downstream signaling pathways like Jak-Stat signaling to interfere with cellular and structural alterations of the pulmonary vasculature.

Therefore, the aim of our study is to determine the extent of JAK-STAT pathway activation in PASMCs from healthy and IPAH patients by Kinome profiling, Western blotting, and BrdU cell proliferation assay.

Results:

Human IPAH patient-derived PASMCs showed a higher Jak kinase activity and mRNA expression, compared to healthy control PASMCs under basal media condition. In vitro proliferation assays revealed anti-proliferative effects of Ruxolitinib by interfering with the altered JAK-Stat3 signaling pathway.

Conclusion:

In IPAH patient-derived PASMCs, aberrant Jak kinase activity, as well as evidence for its involvement in the pathobiology of PAH, suggest that targeting Jak-Stat signaling in PAH would offer new therapeutic strategies.
Abstract No. 530

**LRP1 controls pulmonary artery smooth muscle cell migration and proliferation in pulmonary hypertension**

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Pulmonary hypertension (PH) is characterized by the thickening of the distal pulmonary arteries caused by proliferation and migration of vascular smooth muscle cells. Low density lipoprotein receptor-related protein1 (LRP1) is a scavenger receptor, which regulates platelet-derived growth factor-, transforming growth factor β-, and bone morphogenetic protein-signaling pathways, all known to be involved in the pathogenesis of PH.

We demonstrate the increased levels of LRP1 in lung homogenates and remodeled pulmonary vessels of the patients with idiopathic pulmonary arterial hypertension (IPAH). LRP1 positive staining was observed in media and neointima of IPAH pulmonary arteries. Exposure of primary human pulmonary artery smooth muscle cells (hPASMC) to hypoxia induced LRP1 expression on the mRNA and protein level. Depletion of LRP1 in hPASMC decreased cell proliferation following exposure to hypoxia and enhanced cell migration and adhesion onto fibronectin. Interestingly, these effects were accompanied by the increased expression of β1 integrin suggesting that LRP1 controls hPASMC activities by modulating integrin levels.

In conclusion, our results indicate that LRP1 may regulate hPASMC activities in PH. Thus, LRP1 may be a potential new therapeutic target to prevent remodeling of pulmonary vessels in response to hypoxia.
Abstract No. 531

PEGASUS - the effects of commercial air travel on patients suffering from pulmonary hypertension - a prospective, multicentric analysis

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Background: Patients with pulmonary hypertension on stable background therapy are often able to live a life with only few restrictions. Quality of life includes flights for many people. But the hypobaric hypoxia present during high altitude can have major impact on the medical condition of these patients. Evidence is lacking and the recommendations in the current guidelines are derived from other conditions and other chronic lung disease. We aim to fill this gap of knowledge.

Methods and results: PEGASUS is a prospective, multi-center, multinational observational trial which started in February 2017 and is open until 2019. Primary endpoint is the safety of commercial air travel for patients with PH. In addition, adverse events will be compared with clinical parameters of the patients before they fly to detect possible risk factors. Up to September 2017 180 patients were prospectively included and 17 flights were documented. No adverse event with the need for medical intervention occurred so far. The flying participants had a pulmonary vascular resistance of 5.5 Wood units (median, interquartile range(IQR) 1.9), oxygen saturation at the end of six-minute-walking-test was 93% (IQR 7). 35% use oxygen at home. 6 of the 17 flying patients had a desaturation during flight below 91% measured by pulsoxymeter, three below 86%. 8 patients did not use oxygen while on the airplane and had more severe desaturations down to 78% while the minimum oxygen saturation was 88% in patients using oxygen on board.

Conclusion: We are expecting important safety data for patients with pulmonary hypertension who travel by plane. NCT03051763
Abstract No. 532

Diagnostic accuracy of echocardiography in pulmonary hypertension due to interstitial lung disease

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Background: Pulmonary hypertension (PH) is a common complication in patients suffering from interstitial lung disease (ILD) leading to high morbidity and mortality. Early detection of PH in ILD is essential. The guidelines for detection of PH are mainly based upon publications for other groups of patients at risk for PH. We aimed to evaluate echocardiography as diagnostic tool in ILD-PH.

Methods and Results: We retrospectively analyzed 2D-echocardiography data from patients who underwent right heart catheterization (232 had ILD-PH, 183 with invasive exclusion of PH). Echocardiographic parameters included dimensions, pulmonary artery systolic pressure (PASP), and right ventricular function. The parameters with the highest diagnostic accuracy are PASP 0.839, ICV expiration 0.803, RV diameter basal 0.797, Tei index 0.767, respectively (all p-values <0.001). However, detecting PH in ILD by echocardiography is much harder when compared to other etiological groups (Nizza I, II, and IV). The areas under curves for the strongest diagnostic parameter PASP are 0.935 for CTEPH, 0.930 for PAH, and only 0.839 for LD-PH.

Conclusion: Echocardiography is a good diagnostic tool to detect PH in ILD patients. Especially in end stage or pre-transplant patients this non-invasive tool can easily be used. Even when PASP as the parameter with the highest diagnostic accuracy cannot be obtained, other parameters can help to identify patients in whom right heart catheterization should be considered.
Disease Area End-Stage Lung Disease: Abstract No. 601 – 612
Abstract No. 601

**Fgfr2b signaling in Alveolar Epithelial type 2 cells following pneumonectomy in mice**

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Alveolar myofibroblasts are critical mesenchymal cells for the process of alveologenesis and the formation of the secondary septa. Fgf signaling through Fgfr2b ligands is required for the formation of the alveolar myofibroblasts during alveolar regeneration (Chen et al., 2012). However, the cells receiving such signaling and the mechanism of action of Fgf are still unclear. We showed that during embryonic development, alveolar epithelial progenitor cells respond to Fgf10/Fgfr2b signaling by producing Pdgfa, an essential growth factor for the formation of alveolar myofibroblasts (Ramasamy et al., 2007). We hypothesize that the alveolar epithelial type II cells (AECII) in the adult lung undergo Fgf signaling via the Fgfr2b receptor which is critical for the de novo formation of alveolar myofibroblasts. Moreover, this de novo signaling may be similar to the process happening during the embryonic stages of lung development.

SftpcCreERT2/+;Tomato^lox/+ (control), SftpcCreERT2/+;Fgfr2b^lox/lox;Tomato^lox/+ (experimental) mice are fed with tamoxifen food for one week followed by a chase period with normal food. Sham or left lung pneumonectomy are carried out in control and experimental mice (d0) and the corresponding lungs are analyzed at d4, 7 and 14 during the process of de novo alveologenesis by stereology, IF, FACS (to sort Tomato-positive cells for gene arrays).

Our results with the SftpcCreERT2/+;Tomato^lox/+ mice (PNX vs. SHAM) suggest that Fgf signaling is activated in AECII cells during PNX.

Our ongoing experiments allow us to determine whether Fgfr2b signaling to the AECII cells in the adult lung plays a crucial role during de novo alveologenesis.
Abstract No. 602

IL-17A producing lymphocytes in end stage lung disease

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Background:
IL-17A levels and functional properties have been linked to different chronic lung diseases associated with respiratory failure leading to lung transplantation. In this context, IL-17A secretion (alongside with co-secretion of IL-22) has been mainly attributed to conventional CD3+CD4+ Th17 cells. The contribution of innate lymphocytes (innate lymphoid cells; ILCs, gamma-delta T cells and inducible natural killer T cells; iNKT) to IL-17A and IL-22 secretion in end stage lung diseases remains largely unexplored.

Methods:
We analyzed 14 lymph node (LN) and lung tissue samples derived from lung explants prior to lung transplantation from emphysema and fibrosis patients, respectively, 20 samples from CF patients and 17 LN samples from lung donors as controls. IL-17A and IL-22 secretion was detected by flow cytometry of stimulated (PMA/Ionomycin) single cells suspensions. Supernatants from stimulated single cell suspensions were assessed via multiplex technology to characterize the cytokine patterns associated with IL-17A secreting lymphocytes.

Results:
A significant proportion of IL-17A secretion (22-44%), IL-22 secretion (2-20%) and IL-17A/IL-22 co-production (6-20%) remained unaccounted for by flow cytometric analysis of CD3+CD4+ conventional T helper cells with amounts differing depending on the underlying disease entity. We detected secretion of IL-17A and IL-22 by innate lymphoid cells (lin-IL-7RA+), gamma delta T cells (CD3+gdTCR+) and iNKT cells (CD3+TCRαα24Ja18+). Analysis of supernatants via multiplex technology revealed that IL-17A secretion was accompanied by several IL-17A-associated cytokines and other soluble mediators suggested playing a role in end stage lung disease. Comparison of cytokine patterns revealed significant differences between different disease entities.

Conclusion:
Whilst IL-17A and IL-22 secretion are mainly attributable to conventional CD3+CD4+ Th17 cells, we identified production of these cytokines among populations of ILCs, gamma delta T cells and iNKT cells in different lung disease entities as well, which suggests that these cell populations can contribute to IL-17A-dependent pathologies in end stage lung disease.
Abstract No. 603

**Generation of a NKX2.1/p63 knockin human induced pluripotent stem cell reporter line for monitoring the generation of respiratory cells**

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One promising option to cure hereditary pulmonary diseases like cystic fibrosis might be a cell replacement therapy comprising the generation of patient specific autologous induced pluripotent stem cells (iPSCs), followed by the correction of the underlying genetic mutation, in vitro differentiation into the needed airway epithelial cell type and replacement of the endogenous diseased cells. For long term restoration, most likely lung stem cells like basal cells will be required. A requirement of this strategy is the development of an efficient and robust protocol for the generation of basal cells from human iPSCs (hiPSCs). The transcription factor NK2 homeobox1 (NKX2.1) expressed by lung epithelial progenitor cells represents an appropriate marker for optimizing differentiation protocols towards lung epithelial cells. Combination with the tumor protein 63 (p63) should allow for monitoring of basal cell generation in sequential differentiation protocols. The aim of the present study was the generation of a hiPSC double transgenic reporter line targeting the NKX2.1 and p63 locus. Therefore we designed one targeting vector for a non-disruptive integration of an eGFP coding sequence into the NKX2.1 locus and one targeting vector for the disruptive integration of nuclear localized Venus coding sequence into the p63 locus. Furthermore, the p63 targeting vector introduces a Neomycin selection cassette under control of the endogenous p63 promoter by the use of a 2A-site located behind the Venus coding sequence. The correct integration of the NKX2.1 and p63 targeting vectors was verified using PCR and Southern blot analysis. The established hiPSC-NKX2.1/p63 reporter line represents an optimal tool for the improvement of protocols for the differentiation of hiPSCs into basal cells and enables their selection which is indispensable for further in vitro and in vivo analysis.
Assessment of hollow-fiber membrane oxygenators using computational fluid dynamics

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Extracorporeal membrane oxygenators (ECMOs) have been used clinically for more than four decades as a bridge to transplantation. Hollow fiber oxygenators have gained in popularity since they show low flow resistance and high gas transfer compared to conventional systems. In spite of the advances in extracorporeal technology, the inevitable contact of the circulating blood with the artificial components of ECMOs, and the subsequent thrombus formation, leads to a short-term clinical use (< 30 days). In addition, non-uniform flow can further limit gas-exchange efficiency and influence susceptibility to thrombus formation. Endothelialisation of the hollow-fibre gas-exchange membrane offers a potential solution to ECMO thrombogenicity. However, abnormal shear stresses and inhomogeneous blood flow in the oxygenator could affect the function and activation status of the seeded endothelial cells (ECs).

In this study, the blood flow through a commercially available (MiniLung petite membrane ventilator, Novalung GmbH) and an experimental rodent (RAT device, M.Humbs-Ingenieurburo für Feinwerktechnik, Valley, Germany) hollow-fiber oxygenator was modelled using computational fluid dynamics (CFD), with a view to assessing the magnitude and distribution of the shear stresses on the hollow-fibres and the blood flow fields in these oxygenators. The study developed both heterogeneous, taking into account the full hollow-fiber architecture, and pseudo-homogeneous, considering the hollow-fibers as a porous medium, models. This work showed the need of heterogeneous models in order to predict localized flow fields and shear stresses at the individual fiber level. Specifically, max wall shear stresses ranged between 0.3-2.5Pa according to the clinically relevant flow rates. Moreover, as predicted by CFD models, regions of low blood velocity in the membrane oxygenators were associated with very low shear stress values (< 0.1 Pa) and in areas of recirculating flows. These regions would preferentially match regions with higher incidence of clotting deposition (and long perfusion periods) as well as sites of thrombus formation.
Abstract No. 605

Creating immunologically invisible organs: Silencing MHC expression in an entire porcine lung during normothermic ex vivo perfusion

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Disparities at the loci of the human leukocyte antigen (HLA) remain the main cause for immune rejection and the need for strong immunosuppression after lung transplantation. Recently, we have shown in vivo that MHC-silenced single cells and tissues are able to escape an allogeneic immune rejection. Hence, in this study we evaluated the capacity to silence MHC class I and II expression in the porcine lung. Lentiviral vectors encoding for short hairpin RNAs targeting β2-microglobulin (shβ2m) or the class II transactivator (shCIITA) were produced to target SLA class I or II, respectively. All vectors encoded for NanoLuc luciferase as reporter gene. Lungs explanted from domestic pigs were connected to an ex vivo lung perfusion system and perfused for 2h with the shRNAs encoding lentiviral vectors. Lung endothelial cells (ECs) were isolated, cultured and analysed for bioluminescence. Transcript levels of β2-microglobulin, CIITA or SLA-DR in ECs cultured in presence or absence of IFN-γ were measured by real-time PCR. Histological analyses of the lung tissue were performed. Expression of NanoLuc was already detectable 24h after perfusion in ECs isolated from different lung regions and increased with time. ECs of lungs perfused with shβ2m showed a downregulation of β2-microglobulin by up to 80%. Similarly, lungs perfused with the lentiviral vector encoding for shCIITA induced a knockdown of CIITA, SLA-DR and SLA-DQ by up to 70%. In presence of IFN-γ, ECs from non-perfused lung tissue was able to up-regulate CIITA expression by up to 15-fold. In contrast, levels of CIITA transcripts remained unaffected or even decreased upon IFN-γ stimulation on ECs recovered from lungs perfused with shCIITA. The perfusion and transduction procedure left the integrity of the pulmonary tissue unaffected.

Downregulation of MHC expression creates a status of immunological invisibility and represents a promising approach to combat the burden of rejection and immunosuppression.
Abstract No. 606

**Cytokine expression and alloreactivity in a humanized mouse transplant arteriosclerosis model reflects primary graft dysfunction in lung transplant recipients**

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**Introduction:**

An important cause of early mortality after lung transplantation is primary graft dysfunction (PGD). In a humanized mouse model, we studied the impact of the baseline condition of the endothelium of lung donors in combination with recipient PBMC with or without PGD on the development of luminal occlusion.

**Methods:**

Segments of arteries were implanted into the aorta of immunodeficient mice. Mice were divided into six treatment groups: group A mice with aortic vessels from PGD grade ≥ 2 (PGD+) patients were reconstituted with the respective allogeneic recipient PBMC, group B mice additionally received recipient CD4⁺CD25high Treg cells. Group C mice with aortic vessels from PGD grade ≤ 1 (PGD-) patients received recipient PBMC and group D mice additionally CD4⁺CD25high Treg cells. Control group E mice received arteries from PGD+, group F mice from PGD- patients, both without PBMC reconstitution. Luminal occlusion was quantified histologically and systemic immune responses by quantification of plasma cytokine and chemokines etc.

**Results:**

Luminal occlusion of aortic vessels as sign of TA was significantly more severe in PGD+ group E compared to those from PGD- group F control mice in the absence of PBMC which was paralleled by higher IFN-γ (p<0.05) serum concentrations. Addition of PBMC in group A & C mice further increased luminal occlusion and IFN-γ levels. Treg of PGD+ and PGD- recipients (groups B & D) showed similar suppressive capacity on the development of TA. The IFN-γ-inducible chemokine CXCL10, TNF-α and the soluble high affinity IL-2 receptor confirmed the association of TA with a Th1 response pattern.

**Conclusion:**

We conclude that a pre-existing Th1 response of the donor endothelial system contributes to PGD development, which is further enhanced by alloreactive T cells.
Increased activity of cysteine-proteases on bronchioles and vessels is associated with Bronchiolitis Obliterans Syndrome

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Bronchiolitis obliterans syndrome (BOS) is the most common CLAD phenotype, characterized by mononuclear infiltration and fibrotic tissue deposition around the bronchioles that is associated with a progressive obstruction. The mechanism underlying BOS remains to be defined. Here, we aimed to investigate factors associated with the lung tissue remodeling during the inflammatory response in BOS that affect the survival of BOS patients.

Human BALF, and lung tissue from LTx patients with and without signs of BOS were analyzed by quantitative proteomics, Western blot (WB), protease activity measurements, ELISA, and immunostaining. Orthotopic left lung transplanted (OLLTx) mice were used as model of no-CLAD (syngeneic group) and CLAD (allogeneic group).

Quantitative proteomics of human BALF showed the down-regulation of cystatins (Cys), members of cysteine-protease inhibitor superfamily, in particular CysC (p = 0.0001) and CysS (p = 0.0188), that negatively regulate cysteine-proteases. Cysteine-proteases have been shown to play a role in pro-fibrotic and in pro-inflammatory responses. Furthermore, we pointed out increased activity and amount of cysteine protease in human BALF from BOS patients by WB and protease activity assays, respectively. Histological analysis of human lungs from BOS showed that the accumulation of these proteases in the peribronchial and perivascular compartment were affected by mononuclear infiltration and fibrosis. We confirmed these findings in an OLLTx model for CLAD in mice. In agreement with human outcomes, histological evidence from the CLAD mouse model confirmed the expression of cysteine-proteases in the peribronchial area starting from seven days after LTx, and slightly reduced levels after two months.

In this study we showed that BOS is associated with an increase in the amount and activity of cysteine-proteases. Their accumulation in the peribronchial and perivascular area, that clearly shows signs of fibrosis, justifies further investigation into the role of these proteases in the pathogenesis of BOS.
Nephropathy in patients after lung transplantation – The role of BK and JC Virus

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Chronic renal failure is a major comorbidity after lung transplantation (LTx). Nephrotoxicity is frequently caused by calcineurin inhibitors. In renal transplant patients BK virus (BKV) nephritis is a recognized problem. Untreated BKV infections can cause deterioration of renal function. The aim of this study is to evaluate the role of viral infections in patients after LTx and renal failure. From 2011 to 2016, 119 consecutive patients (66 males, 55.5%) age: 52.6 ± 11.4 were included after LTx (62.2% bilateral LTX). All patients underwent blood and urine sampling before and after LTx (25.3 months ± 33.2 months). We analyzed creatinine, BK and JC virus load by polymerase chain reaction (PCR) in blood and urine samples. One patient underwent renal biopsy after acute drop of renal function. The mean serum creatinine level before transplantation was 0.91 ± 0.21 mg/dl. After LTx (25 ± 33.2 months) serum creatinine level increased to 1.82 ± 1.18 mg/dl. In urine samples, BK virus measured by PCR was positive in 23.5% of the patients (n=28), in serum samples PCR was positive in 3.4% of the patients (n=4). Positivity for JC Virus was found in urine samples by PCR in 21.1% (n=25) of the patients and in serum in 1.7% (n=2) of the patients. One patient with an acute drop of renal function underwent renal biopsy, immunostaining revealed strong positivity for BK virus and BK virus nephropathy was diagnosed. Reduction of immunosuppression and substitution of leflunomide for mycophenolate mofetil was used to treat this patient. Renal dysfunction and the occurrence of BK and JC virus load in patients with high immunosuppression are indicative of possible presence of BK nephropathy in lung transplanted patients. One case with suspicion on BK virus nephropathy could be confirmed by immunohistochemical staining. Further investigations and retrospective stainings of renal biopsy samples are planned.
Abstract No. 609

**Endothelialization of the Ventricular Assist Device Impeller**

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The establishment of a monolayer, consisting of autologous, or immune-tolerable endothelial cells (ECs), on the surface of artificial blood-contacting materials is considered a promising strategy for improving the haemocompatibility of medical devices. With regards to the endothelialization of ventricular assist devices (VADs) that could be potentially used as a drive in a bioartificial lung, the most critical component would be the impeller, where the cells will be subjected to high shear stresses, generated by the rotation of the impeller. In this study we demonstrated that ECs were able to adhere to the surface of the impeller of a VAD (HVAD, HeartWare™), without any prior additional surface coating. Under static cultivation conditions, the ECs grew to confluency and retained a normal physiologic non-activated state.

Moreover, the impeller was modelled using computational fluid dynamics (CFD), with a view to quantifying the magnitude and distribution of the blood-induced shear stresses imposed on its surface, for different physiologically-relevant angular velocities and fluid densities. The EC-monolayer was able to withstand the shear stresses that were generated by different speeds (350, 1000, 1500 and 1800 rpm with an average wall shear stress ranging from 0.4 to 1.4 Pa) in culture medium for 48 h in a customized beaker test setup. Moreover, the cells responded to the stress by up-regulation of genes important for extracellular matrix generation (collagen-IV and Syndecan-2). After pre-conditioning at 350 rpm for 24 h, the seeded impellers were transferred into the original VAD housing and exposed to the shear stress resulting from 1800 rpm, for 20 min. Vital staining revealed several areas covered with an EC-monolayer. The results demonstrate the feasibility to establish a shear-stress adapting EC monolayer on the blood-contacting surfaces of the VAD.
Abstract No. 610

Endothelialization of PMP gas-exchange membranes with human iPSC-derived endothelial cells

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The application of an implantable biohybrid oxygenator, as an alternative to lung transplantation for treating patients with end-stage lung disease, requires the development of long-term haemocompatibility in the device. Biofunctionalization of the blood-contacting surfaces of the oxygenator with autologous or immuno-compatible endothelial cells (ECs) offers a promising approach for inducing complete biocompatibility. The aim of this study was to evaluate the ability of human induced pluripotent stem cell-derived ECs (hiPSC-ECs) to effectively endothelialize fibronectin-coated poly-4-methyl-1-pentene (PMP) gas-exchange membranes used in extracorporeal membrane oxygenators. hiPSC-ECs were seeded onto fibronectin-coated PMP films and analyzed for relevant phenotypic and functional properties after cultivation under static and flow conditions. The results indicated that hiPSC-ECs were able to populate the PMP membranes, while they preserved their major functional properties. Specifically, WST-8 and nuclei counting revealed that the cells maintained their proliferation capacity. Following confluency, the activation, thrombogenic and inflammation status of the hiPSC-ECs was maintained, as evidenced by gene expression analysis and leucocyte attachment testing. Moreover, the integrity of established monolayers was shown by VE-cadherin staining, and their self-healing capacity was visualized by scratch assays. In addition, the monolayers were able to withstand arterial-equivalent flow for 24hrs, while maintaining their major gene expression characteristics. Subsequently, hiPSC-ECs were seeded onto PMP hollow-fiber membranes, where they were able to attach and form a confluent monolayer, as evidenced by VE-cadherin staining. This first proof-of-principle study demonstrated the potential of hiPSC-ECs to biofunctionalize oxygenator gas-exchange membranes. Future in vitro and in vivo studies will evaluate the long-term stability and functionality of hiPSC-EC monolayers on hollow-fiber gas exchange membranes, treated with different surface coatings.
Abstract No. 611

Generation of disease-specific iPSCs and development of transgenic reporter cell lines for cystic fibrosis disease modelling and drug screening

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Cystic Fibrosis (CF) is caused by mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene coding for a cAMP-activated chloride-channel. So far, immortalized cell lines overexpressing mutant CFTR-variants have been used to screen compound libraries. In fact, CFTR-correctors have been identified, but show modest effects at best. Obviously, the complexity of the mutant CFTR-maturation and turnover kinetics including the influence of genetic modifiers require the use of advanced personalized cellular models. To address these unmet needs we focus on the generation and application of induced pluripotent stem cell (iPSC) lines from CF-patients homozygous for p.Phe508del mutation. TALEN-based genome engineering was applied for targeted introduction of reporter transgenes. Several iPSC lines were generated expressing a tomato-fluorescence-reporter under control of one allele of the CFTR-locus. Moreover, a halide sensitive yellow fluorescent protein (eYFP) was introduced into the AAVS1-locus to monitor CFTR-function. To compare the functionality of different CFTR genotypes and CF phenotypes among each other, hiPSC lines were differentiated towards intestinal epithelial cells and analyzed within a microscope-based chloride/iodide exchange assay. The eYFP assay revealed a CFTR channel-specific response in case of functional CFTR expression, which was significantly reduced after treatment with a CFTR-specific inhibitor. Additionally, the functional rescue of p.Phe508del mutated cells via the application of the already known CFTR-modulators VX-770 and VX-809 and/or incubation at low temperature was proven. Results from first experiments in a 384-well format were comparable to the microscopically obtained data and demonstrated a proof-of-concept for the upscaling potential of the CF iPSC-based halide influx assay. The presented project suggests that CF iPSC-derived epithelial cells and the halide sensitive eYFP reporter represent a suitable system for disease modelling in relation to the patient’s geno- and phenotype, that can be used for the conduction of high-throughput (HT) screenings to identify novel compounds for treatment of CF.
Abstract No. 612

High-dose cisplatin application within ex-vivo lung perfusion


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Objectives:

The successful use of ex-vivo lung perfusion (EVLP) in lung transplantation has become reality. Due to the prevention of in-vivo applied high-dose side effects, EVLP turned out as an attractive concept for thoracic oncology, infectious and cell-based research. Here we analyzed the pulmonary impact of high-dose, respectively overdosed cytostatic drug application within EVLP as a supplementary therapy option to treat pulmonary tumors.

Methods:

EVLP was performed for up to four hours in three pig lungs. A combination of blood and low potassium dextran solution was used and added with 300mg, respectively 600mg of cisplatin. At baseline and every hour blood samples were taken for blood gas analysis and immune modulator monitoring, also tissue biopsies and repetitive contrast agent applications for MRI scans were performed.

Results:

Nor cisplatin application, neither repetitive contrast agent application resulted in significant differences with respect to blood gas analysis and histopathological work-ups. It appeared different for immune modulators, as the presence of cisplatin increased the amount of CXCL8, IL-1β, IFN-γ and IL-1RA more than 5-fold at the end of the EVLP procedure, while only a slight increase for IL-6, IL-12 and IL-18 was observed. MRI results showed an increase of permeability parameters (volume transfer constant ktrans) after cisplatin application, indicating an increase in contrast agent influx from plasma into the extracellular space.

Conclusions:

As measured by the in-vivo application, overdosed cisplatin application may be used as an effective supplementary EVLP strategy for tumor therapy. The release of pro- as well as anti-inflammatory immune modulators may enable us to identify novel biomarker candidates for the treatment response to cisplatin in an EVLP approach. Additionally, ex-vivo contrast agent MRI may be used as diagnostic and therapeutic tool without affecting lung function.
Abstract No. 701

**Personalized Treatment of Chemotherapy Induced Anemia**

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**Background:** With 1.8 million of new cases per year, lung carcinoma is globally the most prevalent cancer with the highest incidence. The prevalence of anemia associated with lung carcinoma ranges from 50% to up to 90% in the most advanced stages. Two therapeutic options are available to manage chemotherapy induced anemia, blood transfusions and/or erythropoiesis stimulating agents (ESAs). For both options adverse events and increased risk of mortality have been reported, and the guidelines recommend limiting the use of both approaches to the minimum required quantity. The clinical decision is based on the benefit-to-risk ratio in each patient and the complexity is due to the heterogeneity of the patients, the lack of prognosis markers and the dynamics of comorbidities related with the disease.

**Results:** We developed a mechanistic mathematical model to guide clinical decisions based on the prediction of the response to the available therapeutic options. The mathematical model stratifies patients based on the estimation of two dynamic patient specific parameters. These parameters are estimated by the mathematical model based on the time-course of the Hb, CRP values and scheduled chemotherapy in each patient. These two patient specific parameters reflect the anemic status of the patient as well as the capability to respond to treatment with ESAs.

**Conclusion:** The model is capable to propose optimized personalized interventions in anemia management in lung cancer patients. The model recommends the patient-specific safer minimal effective dose of ESA and/or blood transfusion.
Abstract No. 702

KrasG12D-Mad2-driven Aneuploid Lung Adenocarcinomas are Characterized with Higher Immunosuppressive Phenotype than Their Euploid Counterparts

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Efficacy of Immune Checkpoint inhibitors (ICI) in the treatment of Non-Small Cell Lung Cancer varies widely and only a subset of patients respond to therapy. The identification of reliable biomarkers that predict response to ICI is nowadays a major need.

Recent studies have found that tumors with high mutational burden predicted to express more neoantigens responded with a more robust immune response than tumors containing low number of mutations. We reasoned that since aneuploidy is a common feature of solid tumors, the levels of aneuploidy in a tumor could potentially also modify treatment response to ICI.

To interrogate the possibility that immune cells recognize aneuploid tumor cells in vivo, we used an aneuploid lung adenocarcinoma model, in which mutant Kras (K group) and Mad2 (KM group) are overexpressed in type II pneumocytes. The survival curve of these mice show a statistically significant decrease in survival in KM versus K mice. In order to investigate the involvement of the immune system in this phenomenon, we analyzed the immune landscape of the lungs in both groups.

These data show no significant differences in B cell infiltration between K and KM groups, but ~4 times more CD3+ T-cells were noticed in KM versus K tumors alone. Detailed analyses of CD3+ T-cells shows higher infiltration of KM nodules with CD4+ lymphocytes, whereas CD8+ cells’ infiltration was not different in both groups. Additionally, aneuploid tumors (KM group) are significantly more infiltrated with tumor associated macrophages (TAMs) compared to euploid tumors (K group). We also found, that KM lungs have more Tregs and less NK cells.

Altogether, this preliminary data give us a hint to think that the immune microenvironment in aneuploid lung tumors is more immunosuppressive than in euploid controls. This difference in the immune microenvironment suggests that the treatment responses to ICI may also be different.
Abstract No. 703

Synthetic lethality of KRAS-mutant tumor cells in vivo depends on inflammatory signaling to myeloid cells

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Introduction: KRAS-mutant cancers are notoriously undruggable and in vitro drug screens are unfruitful in identifying novel anti-KRAS agents. Molecularly-designed KRAS inhibitors have recently emerged, but not been characterized.

Objectives: We determined the efficacy of KRAS inhibitors against tumors with/without KRAS mutations in vitro and in vivo to identify resistance mechanisms.

Methods: We treated tumor cells with defined KRAS mutation status with KRAS inhibitors deltarasin, cysmethynil, and AA12. KRAS silencing and overexpression were done using shRNA and KRASG12C vectors coupled with microarray analyses. Mice sufficient or deficient in interleukin (IL)-1β (Il1b−/−) or chemokine receptors (Ccr2−/−, Cxcr1−/−, Cxcr2+/−) received s.c. KRAS-mutant or wild-type tumor cells followed by saline or deltarasin treatments.

Results: We identified that KRAS inhibitors exerted comparable effects against cancer cells in vitro irrespective of KRAS status. However, mice with KRAS-mutant tumors responded selectively to deltarasin treatment, in contrast to mice with KRAS-wild-type tumors. Similar in vivo-restricted effects were evident after genetic manipulation of KRAS. Microarrays identified a 42-gene signature specific to KRAS-mutant cells and responsive to KRAS manipulation, which contained Kras, Ccl2, Il1r1, Ccl7, and Cxcl1. Deltarasin was effective in halting KRASG12C-mutant flank tumors in Wt, Cxcr1−/−, and Cxcr2+/−, but not in Ccr2−/− and Il1b−/− mice.

Conclusions: Inflammatory signaling loops are synthetic lethality targets for KRAS mutant tumors and only druggable by KRAS inhibitors in vivo. Hence in vitro drug screens may be suboptimal settings for anti-KRAS drug discovery.
Abstract No. 704

**A high throughput whole animal screening system for lung cancer therapeutics based on tailored Drosophila models**

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Lung cancer is one of the most aggressive types of cancer and became the leading cause of cancer deaths. As the survival rate in non-small cell lung cancer is extremely poor, alternative, more effective therapeutic interventions are desperately needed. In order to identify novel small compound based therapies, we used *Drosophila* to establish a whole animal based high throughput screening system. The airway system of *Drosophila* shares comprehensive structural and functional similarities with the human lung, thus providing the possibility to study the molecular framework underlying lung cancer development. We could show that ectopic overexpression of several oncogenes induced cancer-like phenotypes in the *Drosophila* airway system, comprising meta- and hyperplasia of airway epithelial cells. One of the key oncogenes in lung cancer was selected for an in-depth characterization of lung cancer-like phenotypes and the development of a high-throughput screening system. Ectopic activation of the EGFR pathway in the airway epithelium by expression of a constitutively active EGFR isoform leads to thickening of the epithelium and increased numbers of cells indicative for epithelial meta- and hyperplasia. This intervention led to early death due to oxygen undersupply. We used this lethal phenotype to develop a high-throughput drug screening system approach based on rescuing this lethality. By screening a FDA-approved drug library with about 1000 compound, we found that the EGFR inhibitors Afatinib, Gefitinib and Erlotinib were the only compounds that rescued lethality. The treated EGFR larvae were able to survive the larval stages and successfully developed into pupae and adults. Epithelial meta- and hyperplasia could be completely rescued by reversing epithelial hyper- and metaplasia. In the future, we will employ this system to identify novel compounds and combinations of compounds that could rescue this lethality without interfering with developmental processes or the normal physiology.
Abstract No. 705

**Active chitinases as potential targets for lung cancer therapy**

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Lung cancer causes the largest number of cancer-related deaths worldwide. More than 85% of lung cancers are currently classified as non-small-cell lung cancer (NSCLC), with a low predicted 5-year survival rate. New treatment strategies are available; however, responses are typically short-lived. Therefore, there is a demand for the development of more efficient therapeutic regimes. One of the potentially effective approaches to treat cancer is to target both tumor cells and tumor-stroma components, including vasculature, stromal fibroblasts, and immune cells. To assess novel strategies for lung cancer treatment, we investigated a Kras-driven mouse model of NSCLC, orthotopically-grown primary human lung cancer and clinical material from the patients with lung adenocarcinoma. We found that tumor progression in mouse models of lung cancer correlated with overproduction of secreted lectins – active chitinases – in blood plasma and in the lung tumor lesions. Similarly, patients with lung adenocarcinoma showed increased chitinase activity in blood plasma and greater AMCase staining of fixed cancer specimens. We demonstrated that AMCase chitinase was produced by tumor-associated macrophages as one source for active chitinases in the lung cancer stroma. We propose that chitinase-dependent macrophage functions play a critical role in driving lung tumorigenesis and that blocking this process could have therapeutic potential.
Abstract No. 706

**Interferon Regulatory Factor 9 Mediated Regulation of Lung Cancer Progression and Metastasis**

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Lung cancer is the leading cause of cancer-related death worldwide and accounts for more than 1.6 million deaths per year. The tumor microenvironment was shown to play a crucial role in tumor progression and metastasis. Beside numerous cytokines, chemokines and other factors secreted by the tumor stroma, Type-I-IFNs are strong immune modulators, showing antiproliferative and proapoptotic properties. We aim to study the essential role of the transcription factor IRF9 (Interferon Regulatory Factor 9) in the IFN-pathway in lung cancer.

Based on Kaplan-Meier estimators, high levels of IRF9 in lung cancer patients are associated with a significantly lower survival. Using tissue microarrays we could show that IRF9 is expressed in most of the lung cancer entities. In human lung cancer tissues IRF9 is expressed in both, the solid tumor part and the tumor stroma, where we identified strong expression of IRF9 in dendritic cells and tumor-associated macrophages. *In vitro* we used lentiviral particles to transduce the adenocarcinoma cell line A549 to stably overexpress (A549 LV IRF9) or to stably suppress IRF9 (A549 shIRF9). A549 LV IRF9 show an increase in proliferation and migration, whereas the knockdown of IRF9 leads to a reduction in proliferation and migration. In addition, these findings were confirmed in a subcutaneous *in vivo* xenograft model, where increased (A549 LV IRF9) and accordingly decreased (A549 shIRF9) tumor sizes were observed.

These results suggest that IRF9 is one important transcription factor to target cancer and microenvironmental cells in lung cancer therapy.
Abstract No. 707

Risk of lung adenocarcinoma from smoking and radiation arises in distinct molecular pathways

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SUMMARY

Lung adenocarcinoma (LADC), the deadliest cancer worldwide, is mainly caused by tobacco smoke and radiation that cause mutations in the KRAS, EGFR, and other proto-oncogenes. While these mutations are found in different frequencies in Caucasian and Asian LADC patients and while KRAS mutations are more frequent in smokers, other risk factor-oncogene links are unknown and molecular risk prediction models are missing. We reanalyzed published genomic data to identify two broad molecular pathways to LADC and used unique information from the Life Span Study of Japanese atomic bomb survivors to develop a molecular mechanistic model of LADC risk prediction. The new model accurately predicts the incidence of LADC stratified by risk factor and molecular pathway, confirms the existence of the two pathways, explains population differences in driver mutation frequencies, and provides the first direct epidemiologic linkage of risk factors smoking and radiation with the two molecular classes of LADC.

SIGNIFICANCE

We developed the first mechanistic model for risk prediction of lung adenocarcinoma stratified by molecular driver pathway. The model can explain how smoking and irradiation contribute to different molecular classes of lung adenocarcinoma in Caucasian and Asian populations.

Key words: KRAS; EGFR; molecular pathway; radiation; smoking; mechanistic model; driver mutation; oncogene; non-small cell lung cancer.
Abstract No. 708

Defining the cell of origin of lung adenocarcinoma via transcriptome and methylome analysis

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The identity of the lung adenocarcinoma cell of origin and its contributions to disease progression and treatment response remain largely unknown. To unveil the cell of origin of lung adenocarcinoma (LADC), we are using tamoxifen-inducible Cre-ER strains (Scgb1a1.CreER, Hoxp-CreER, Sftpc.CreERT\textsuperscript{2}-rtTA, K5.CreER) to mark specific adult cell lineages in combination with the mT/mG reporter mouse. These models allow the labelling of different types of lung epithelial cells (Clara cells, alveolar type I and II cells) as well as basal cells, all of which could contribute to LADC inferred from published data. To induce LADC in mice, we have established a CRISPR/Cas9 based EML4-ALK rearrangement and a N-Nitroso-N-methylurea (MNU)-induced lung cancer model. Both models successfully develop LADC stochastically and highly resemble the nature of human LADC, in contrast to previous studies where tumors were induced by forcefully expressing an oncogene in one cell type.

Although current studies have identified molecular modifications occurring during lung cancer initiation, the upstream genetic and epigenetic alterations of LADC are still unknown. By finding out the unique signatures in DNA methylation and gene expression, this study will provide both cellular and genetic evidences of lung cancer origin that will benefit the development of novel therapies for human lung cancer.

Preliminary results from the in vivo mouse models and FACS sorting techniques will be presented during the meeting.
Abstract No. 709

**EML4-ALK fusion variant V3 is a high-risk feature in ALK+ NSCLC**

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**Purpose:** Sequential administration of tyrosine kinase inhibitors (TKI) combined with local ablative treatments has considerably improved the prognosis of stage IV ALK+ NSCLC patients with a median overall survival (OS) currently exceeding 5 years after two ALK inhibitors. An unmet need is the identification of patients with worse outcome, who may benefit from different approaches.

**Methods:** Included in this retrospective analysis were all patients treated at our institutions for metastatic lung adenocarcinoma bearing one of the main EML4-ALK fusion variants V1, V2, V3 as detected by NGS or RT-PCR.

**Results:** Sixty-seven patients could be identified with median age of 59 years (interquartile range [IQR] 19) and a median ECOG performance status of 0 (IQR 1). Most prevalent was variant V3 (51% of cases), followed by V1 (39%) and V2 (10%). Patients with V3 tumors had more metastatic sites at diagnosis than cases with the V1 and V2 variants (mean 3.3 vs. 1.9 and 1.6, p<0.05), which suggests increased disease aggressiveness. Furthermore, V3+ status was associated with earlier failure after treatment with ALK TKI (median progression-free survival [PFS] in the first line 219 vs. 1179 days, p=0.01) and platin-based combination chemotherapy (median PFS 162 vs. 456 days for the first line, p<0.01), and with shorter overall survival (median OS 1195 vs. 1789 days, p<0.05).

**Conclusions:** EML4-ALK variant V3 is a high-risk feature for ALK+ NSCLC. Determination of V3 status could be considered as part of the initial workup for this entity in order to select patients for more aggressive surveillance and treatment strategies.
Abstract No. 710

Receptor turnover and transcriptional negative feedbacks control cell type-specific dynamics of the TGFβ pathway in lung cancer

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Rationale: Non-small-cell lung cancer (NSCLC) is the leading cause of cancer-related mortalities worldwide. Elevated levels of the Transforming Growth Factor-beta (TGFβ) ligand correlate with a poor survival of lung cancer patients. However, the regulatory mechanisms controlling pathway activation in lung cancer remain poorly understood.

Aim: In the presented work we combined quantitative data generation and mechanistic mathematical modeling to understand how the dynamic properties of the pathway are controlled.

Methods: We measured the time-resolved dynamics of TGFβ-induced Smad2/3 phosphorylation by quantitative immunoblotting. Inhibitors of mRNA transcription, protein translation or protein degradation were used to perturb the signaling network. The depletion of the TGFβ1 ligand from the cell culture medium was determined with a bead-based assay. The expression of transcriptional negative feedback regulators was assessed by qRT-PCR. siRNA-mediated knockdowns were performed to examine the importance of different feedback regulators. The degree of Smad2 phosphorylation and the abundance of the TGFβ-receptor were assessed by quantitative mass spectrometry.

Results: The analysis of TGFβ-induced signal transduction in three NSCLC cell lines showed a distinct dynamic behavior of the TGFβ-induced Smad2/3 phosphorylation and a differential impact of inhibitor perturbations. These results suggested a differential prevalence of negative feedback regulators that induce the degradation of the TGFβ-receptor or reduce its ability to phosphorylate Smads. The model-based analysis predicted that the TGFβ-receptor undergoes constant turnover: the unstable receptor is constantly degraded and produced again from stable mRNA. We experimentally confirmed a high stability of the TGFβ-receptor mRNA, while the accumulation of the TGFβ receptor protein upon inhibition of the proteasome function was validated using targeted quantitative mass spectrometry.

Conclusion: These findings highlight that the TGFβ receptor is one of the most sensitive nodes that controls pathway activation. Therefore, targeting processes that control receptor abundance rather than using conventional TGFβ kinase inhibitors could be a promising therapeutic approach.
Abstract No. 711

**Secretion of Soluble Factors by H1975 Lung Adenocarcinoma Cells Influence on Na-Transport in Human Alveolar Epithelium**

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RATIONALE: A lung tumor microenvironment consists of cells surrounding the tumor and substances released by the tumor or adjacent tissue. None of these surrounding cells appear malignant yet; however interaction with the tumor may cause abnormal phenotypes and functions. We hypothesize that there is interaction between lung cancer cells and healthy alveolar epithelium, and that neighboring cancer cells modify ion-transport of healthy tissue.

METHODS: Primary human alveolar epithelial cells (hATII) were isolated from the tumor free regions of human lung tissue after resection. Confluent hATII monolayers were treated with conditioned media obtained from normoxic and hypoxic H1975 cells. For the characterization of possible signaling molecules, conditioned media were also fractionated by filtration with different cut-offs (>10 kD, >30 kD, and >50 kD). It was also studied whether signaling molecules are contained in vesicles released from H1975 cells, where vesicles were collected using a commercial isolation kit. hATII were also treated with the Gsα-antagonist NF 449 and the PKA inhibitor H-89 to test for the involvement of G-protein coupled receptor and cAMP signaling. hATII cell function was evaluated by measuring ion transport (epithelial Na channels, Na/K-ATPase) in Ussing chambers.

RESULTS: H1975 conditioned media stimulated ENaC and Na/K-ATPase whereas hypoxic H1975 conditioned media decreased Na-transport. The stimulatory effect of conditioned media was lost after removing molecules >10kD, but stimulation was fully present in conditioned media after excluding molecules >50 kD. NF 449 and H-89 prevented stimulation of Na-transport by conditioned media.

CONCLUSIONS: These results demonstrate that H1975 adenocarcinoma cells release soluble factors that stimulate hATII cell Na-reabsorption, which is mediated by G-coupled receptor signaling, and that these factors have a molecular weight between 10 kD and 50kD and are not contained in the exosome fraction. Stimulatory factors are not released from hypoxic H1975.
Abstract No. 712

Endotoxin induces resistance to radiotherapy in Non-Small Cell Lung Cancer Cell Lines in vitro – role of EGFR and CREB

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RATIONALE: Lung cancer is the leading cause of cancer death. Pulmonary infections are common complications in patients with lung cancer and worsen prognosis. These patients often show an acquired resistance to radiotherapy. Gram-negative bacteria are common pathogens in lung cancer. Their virulence is caused by cell wall components, especially by Lipopolysaccharides (LPS). LPS is known to activate multiple pathways in pulmonary epithelial cells. It has been shown that LPS promotes non-small cell lung cancer (NSCLC) growth in vitro an in vivo. It is not clear whether LPS could induce radiotherapy resistance in NSCLC cells. METHODS: NSCLC cell lines with different epidermal growth factor receptor status (EGFR) were pre-incubated with LPS and then exposed to ionizing radiation. The clonogenic survival was quantified by a colony formation assay. In parallel, proteome arrays were performed. Up-regulated target proteins were inhibited in LPS-treated cells before irradiation. RESULTS: Ionizing radiation induced a reduction in clonogenic survival. However, in LPS treated cells the effect of ionizing radiation was severely attenuated; the sensitivity to radiotherapy was remarkably decreased in the human adenocarcinoma cell line H1975. This effect was dose dependent and most pronounced when 10 µg/ml LPS were used. In H1975 cells the survival fraction increased significantly in the presence of LPS. The proteome array shows an upregulation of the cAMP response element-binding protein (CREB) and EGFR after LPS treatment and radiation. After CREB binding protein (CBP) or EGFR inhibition the LPS-induced resistance to radiotherapy was decreased, meaning sensitivity to irradiation was restored. CONCLUSION: The LPS treatment of H1975 cells induces radiotherapy resistance. Therefore, inhibition of possible target proteins like CREB or EGFR may serve as a potential treatment to overcome resistance to radiotherapy.
Abstract No. 713

The role of long non-coding RNAs in tumor-associated macrophages

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The tumor microenvironment has a great impact on lung tumor progression, metastasis and angiogenesis and it was shown that tumor-associated macrophages (TAMs) act as key regulator cells. The molecular pathways underlying this regulation are however still not fully understood. This study explores the role of long non-coding RNAs (lncRNAs) in macrophage polarization in context of the tumor microenvironment.

To identify deregulated lncRNAs in distinct macrophage phenotypes, RNA Sequencing was performed from LPS + IFNγ stimulated M1 and IL-4 stimulated M2 human macrophages which revealed a high number of aberrant expressed lncRNA-genes in the two phenotypes. We selected two novel lncRNAs that we named MSLRA1 and MSLRA2, which were strongly upregulated in M2 macrophages. These lncRNAs were almost exclusively expressed in macrophages as well expression increases during monocyte to macrophage differentiation (day 1-10). In addition, subcellular fractionation showed that MSLRA1 was localized predominantly in the nucleus whereas MSLRA2 was present in both, nucleus and cytoplasm. Interestingly, knock down (KD) of lncRNAs using antisense LNA GapmeRs in M2 macrophages led to an increased expression of different M1 marker genes like IL-8 or TNFα and downregulation of M2 marker expression like CSF1R or IL10, suggesting a function in maintaining the M2 phenotype. To understand the tumor promoting capabilities in vitro, lncRNA-silenced macrophage conditioned medium (CM) led to changes in tumor cell functional assays. Particularly, MSLRA2 KD-M2-macrophage CM lead to decreased tumor cell migration and MSLRA1 KD-M2-macrophage CM lead to increased apoptosis in tumor cells like M1 macrophages function. However, both lncRNAs KD-M2-macrophage CM had no effect on tumor cell proliferation. Similarly, lncRNA KD-CM on endothelial cells resulted in decreased tube formation.

These results show the impact of lncRNAs on macrophage polarization and tumor progression and suggest that lncRNAs might serve as a therapeutic target in lung cancer treatment.
Abstract No. 714

Biomarkers of platelet activation in relation to lung cancer risk

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Background: Enhanced platelet activation and a pro-coagulative state are related to worse prognosis among lung cancer patients. Experimental data further suggest that platelet activation may drive tumor initiation and promotion, but prospective human studies are missing. Preliminary data from clinical case-control comparisons also suggest that markers of platelet activation could be used for prediction of lung cancer risk and survival.

Methods: Pre-diagnostic plasma concentrations of platelet activation biomarkers (P-Selectin, Glycoprotein IIb/IIIa, Thrombopoietin, Thrombomodulin, and Fibrinogen) were measured in a case-cohort subset of the population-based EPIC-Heidelberg Study (sub-cohort: n=1185; incident cases: n=160; all ever-smokers). Associations between biomarker levels and lung cancer risk were evaluated by Cox regression models, adjusting for all risk factors that are part of the established PLCOm2012 risk prediction model, i.e. age, sex, smoking duration, smoking intensity, COPD, BMI, education, and cancer history.

Results: P-Selectin (Hazard Ratio: 4.98 [95 % Confidence Interval: 2.91, 8.50]) and Fibrinogen (HR: 3.85 [2.32, 6.37]) were significantly associated with increased lung cancer risk in age- and sex-adjusted Cox regression models. Upon multivariable adjustment, the associations were attenuated but remained statistically significant, with HRs of 2.77 [1.55, 4.96] for P-Selectin and 2.03 [1.16, 3.55] for Fibrinogen. Adding both markers to the PLCOm2012 algorithm, which alone showed an area under the receiver operator curve (AUROC) of 0.788, slightly improved lung cancer risk prediction (AUROC=0.814). The respective net reclassification improvement (NRI) with P-Selectin and Fibrinogen in addition to the PLCOm2012 algorithm was 0.17 (p=0.015). Statistical adjustment for CRP levels only marginally affected the reported associations.

Conclusion: P-Selectin and Fibrinogen are significantly associated with lung cancer risk and may modestly improve risk stratification among former and current smokers. Despite independent associations of P-Selectin and Fibrinogen with lung cancer risk, both markers were positively correlated with smoking status, and it is conceivable that they partially mediate smoking-induced carcinogenesis.
Subclonal tree clustering of pulmonary adenocarcinoma based on spatially distributed somatic mitochondrial mutations

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Introduction Targeted therapies of non-small cell lung cancer based on tissue-derived biomarkers propelled the concept of lung cancer specific therapies. Although 5-year survival has improved considerably, pulmonary adenocarcinoma (ADC) commonly develop resistance and relapse. One clue to this tenacity is intratumor heterogeneity: a subclonal evolution can cause non-uniform characteristics of a tumor.

In a previous study (Kazdal et al. 2017 in BJC) we could show that somatic mitochondrial mutations have a great potential to be used as progression markers in NSCLC. In order to further evaluate this we systematically analyzed the occurrence of somatic mitochondrial mutations in the complete expansion of central sections of ADC.

Methods Following a systematic segmentation of central sections of 19 ADC (11-34 segments with a dimension of 5 x 5 mm per tumor) the following analysis were performed for each segment separately as well as for corresponding samples of non-neoplastic tissue and lymph node metastasis if applicable:

- Histomorphological characterization
- Determination of the tumor cell content (TTF1-IHC, digital pathology)
- Total DNA and mitochondrial DNA quantification (QuBit, qPCR)
- Detection of somatic mitochondrial mutations (whole mitochondrial genome panel sequencing)

Based on the somatic mitochondrial mutations phylogenetic tree clusters were calculate for each tumor section.

Results & Discussion Somatic mitochondrial mutations could be detected in 16 (84%) of the analyzed ADCs. Mutation were either present in all tumor segments or restricted focally to specific tumor regions indicating a subclonal development of the respective tumor. The detection of regional restricted mutations was only possible due to the systematic analysis of complete tumor sections and would have failed in a single region based analysis. The phylogenetic clustering illustrates the relative distance of the segments to each other and to non-neoplastic tissue of each tumor. Hence, this approach indicates regions with differing progression levels within a tumor.
Abstract No. 716

**Comprehensive clinical profiling of the Gauting locoregional lung adenocarcinoma donors**

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**Rationale:** Lung adenocarcinoma (LADC), the leading cancer killer, is emerging as a distinct disease entity. However, a comprehensive characterization of its clinical features is missing.

**Objectives:** To prospectively evaluate the phenotype and prognosis of Caucasian patients with early-stage LADC in the Gauting lung adenocarcinoma donors (GLAD) study.

**Methods:** Patients with LADC diagnosed between 2008 and 2015 were prospectively assessed for lung resection with curative intent. Fifty-five clinical, pathologic, radiologic, and molecular variables were recorded. Patients were followed till death or study conclusion. The main findings were validated in a separate prospective cohort from Tours, France.

**Measurements and Main Results:** Of 1943 patients evaluated (28% of registered Bavarian cases over the study period), 455 were potentially resectable and 366 were enrolled (18.8%; 181 female; 75 never-smokers). Smoking and obstruction were significantly more prevalent in GLAD compared with adult Bavarians ($P < 0.0001$). Ever-smoker tumors preferentially localized to the upper lobes. Surgery rendered 301 patients (82.2%) tumor-free at the 30-day-post-surgery benchmark, of which 99 relapsed and 74 died over 704 cumulative follow-up years. Median overall and disease-free survival was > 7.5 and 3.6 years, respectively. Patients aged < 45 or > 65 years, resected > 60 days post-diagnosis, with abnormal FVC/DLCOVA, N2/N3 stage, or solid histology had significantly decreased Kaplan-Meier ($P < 0.05$) and increased Cox proportional hazard ($P < 0.01$) survival estimates. These were fit into a weighted LADC death risk score (LADERS) that outperformed cTNM/pTNM7 in predicting survival in the GLAD derivation and Tours validation cohorts.

**Conclusions:** We define the clinical gestalt of locoregional LADC and provide a new simple and feasible prognostic tool, findings that may aid future management and research design.

**Key words:** non-small cell lung cancer; smoking; obstruction; survival; mutation.
Abstract No. 717

Modulation of cancer cell growth and tumor-microenvironmental factors in ex vivo co-cultures of fresh human lung tissue and patient-derived, disseminated cancer cells

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The transgression towards new organs as part of the invasive metastasis cascade marks the main cause of death in most cancers. The complexity of this process remains challenging for most preclinical models due to high cellular and genetic disparities between primary tumor and metastasis. Most models furthermore lack the native human microenvironment, leading to less predictive test systems.

Metastatic tumor growth is here modulated within its natural microenvironment by adding patient-derived, disseminated and GFP-labeled melanoma cells allogeneically to human ex vivo lung slices. Effects on tumor growth reduction and modulation within its natural microenvironment in solid tumors were determined by preparing fresh human tumor tissue slices. Cancer cells integrate into the lung tissue and proliferate (6fold increase of cell number within the first 24 hours of co-culture). The cancer cell number decreases to initial levels 72 hours after seeding but recovers after 120 hours (5.4fold increase). Macrophage infiltration through CD68 staining was observed, with a 19fold increase of CD68-positive cells in the cancer cell afflicted areas after 120 hours. Macrophage-cancer cell interactions were seen at all time points with accumulations of melanoma cells not interacting with macrophages being observed after 96 hours, hinting at selective processes with these cells growing out after 120 hours. Treatment with Vemurafenib on tissue invaded by melanoma cells carrying the V600E mutation that causes sensitivity towards treatment led to a 71% decrease of cancer cells after 48 hours using the highest concentration of 50µM whereas non-mutated cells showed no significant cancer cell decrease.

Here we modulate cancer cell proliferation, growth and mediator concentrations in human lung tissue, showing that tumor cells in solid tumors and freshly seeded into healthy lung tissue are sensitive to tumor treatments ex vivo. We propose this model as a new option for preclinical drug testing in oncology.
Abstract No. 718

SERPINA1 gene expression in lung cancer cells and its role in cell apoptosis

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Alpha1-antitrypsin (AAT) is an acute phase protein and a major inhibitor of human serine proteases. We previously published that plasma levels of AAT are significantly higher in newly diagnosed lung cancer patients. Cancer patients with high levels of proteinase inhibitors are often characterized by a more disseminated disease and worse prognosis. Regarding the relationship between the levels of AAT and cancer progression, data are contradictory. Recent findings have linked increased AAT levels with suppression of intrinsic apoptosis pathway via activation of PKC/Akt pathway and the myeloid cell leukemia 1 protein accumulation. Therefore, we postulate that the level of AAT expression in various lung cancer cells may contribute to cell resistance against apoptosis. Our preliminary results show that SERPINA1 gene expression differs considerably across lung cancer cells at the baseline. Among cell lines that we studied, NCI-H1563, NCI-H1975, and NCI-H1299 showed the highest whereas NCIH661, NCI-H2126, NCI-H1437, and NCI-H1573 cells showed the lowest SERPINA1 expression. Baseline levels of B-cell lymphoma 2 (BCL-2) gene, a member of the Bcl-2 family of anti-apoptotic proteins, were also higher in NCI-H1563, NCI-H1975, and NCI-H1299 cells while NCI-H661, NCIH2126, and NCI-H1437 demonstrated much lower BCL-2 expression. Upon stimulation with bacterial lipopolysaccharide (1μg/ml), all studied cancer cells decreased expression of SERPINA1 gene as well as BCL-2. These findings encourage further studies on the regulation of expression of SERPINA1, BCL-2 and other anti-apoptotic genes in lung cancer cells.
Abstract No. 719


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Background: The survival of lung cancer (LC) patients strictly depends on the stage and hence the timepoint of diagnosis, therefore an early detection is crucial. CT based screening protocols have been shown to be efficient in lowering mortality in LC, but at the price of a high number of false positives. Therefore, there is a high unmet clinical need to improve screening and increase specificity by addition of non-invasive tests. The electronic nose technology is of longstanding interest for detection of various diseases and offers for such purpose. We analyzed the potential use of electronic nose (Aeonose®), as a simple, non-invasive tool for principle detection of LC.

Methods: In total, signatures of exhaled volatile compounds (VOCs) of 30 incident and untreated prevalent LC patients, who did not smoke in the last 2hrs prior to measurement, were captured via Aeonose® and compared to 25 healthy subjects. Area under Curve (AUC) and Matthews correlation coefficient (MC) were used to interpret the Aeonose® data.

Results: The Aeonose® was clearly able to distinguish between patients with LC and healthy subjects (n=25), showing an AUC of 0.95 and MC of 0.85.

Conclusions: The Aeonose® shows promising potential in safely identifying a mixture of different LC cases (incident – prevalent; different histologies). Additional studies are needed to further proof the sensitivity and specificity of this approach in LC patients.
Abstract No. 720

Tumor-Derived Granulocyte Chemotactic Protein 2 Cooperates with Neutrophil Proteinase 3 To Drive Lung Adenocarcinoma

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Introduction: Lung adenocarcinoma (LADC) commonly arises in the lungs of smokers that are heavily affected by chronic inflammation. Inflammatory signaling from tumor to host cells is critically involved in the pathogenesis of LADC, but the exact mechanisms by which the lung epithelium interacts with the immune system during carcinogenesis are obscure.

Objectives: We discovered that murine and human LADC cell lines overexpress the inflammatory and angiogenic CXC chemokine granulocyte chemotactic protein 2 (GCP2) compared with normal epithelial cells and aimed at investigating its function(s).

Materials and Methods: GCP2, neutrophil elastase (ELANE), and proteinase 3 (PR3) expression were determined by ELISA and immunohistochemistry. Mouse and human microarray data were analyzed using Affymetrix Transcriptome Analysis Console. GCP2 silencing using dedicated shRNA pools (SantaCruz Biotechnology) and ELANE/PR3 compound knockout mice were used to study GCP2 interaction with neutrophil serine proteases in LADC progression.

Results: Murine and human LADC tumors and cell lines overexpressed GCP2 and ELANE/PR3 at the mRNA and protein levels. GCP2 was sequestered to tumor cells, whereas ELANE/PR3 were produced by tumor-infiltrating neutrophils. LADC-secreted GCP2 was incompletely processed and required ELANE/PR3 for full activation and vasoactive effects. Both GCP2-silenced LADC cells and ELANE/PR3-deficient mice were protected from LADC progression.

Conclusions: Our results indicate that tumor-originated GCP2 cooperates with neutrophil ELANE/PR3 to drive LADC, providing a paradigm of how the respiratory epithelium coopts the innate immune system during carcinogenesis.

Acknowledgements: This work was supported by the European Research Council.
Abstract No. 721

**Exosome RNA-Seq Analysis To Isolate Lung Cancer Specific Pseudogenes For Liquid Biopsy**

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**Background:**  
Recently, it has been reported that several exosome non-coding RNAs are overexpressed in non-small-cell lung cancer (NSCLC), and have been identified as key regulators in post-genomic biology; moreover it also has been suggested as potential biomarkers for a non-invasive “liquid biopsy” in cancers’ diagnosis and prognosis. In this study, we screened for human NSCLC associated pseudogenes in plasma exosomes.

**Methods:**  
13 lung adenocarcinoma cancer patients and 15 health volunteers were recruited. We characterized non-coding RNA transcripts by using RNA-seq analyses of ribosomal RNA-depleted total RNA from the plasma exosomes. DESeq2 package was used to identify differentially expressed non-coding RNAs after raw data filtering. The patients’ exosome pseudogenes were enriched at least 2-fold compared with normal control.

**Results:**  
An expression of eight pseudogenes were found to be associated with lung adenocarcinoma cancer patients.

**Conclusions:**  
This study indicates that the pseudogenes can be detected in cancer patients’ plasma exosomes, and eight-pseudogenes signature may serve as an effective non-invade “liquid biopsy” prognostic molecular biomarkers in NSCLC patient.  
Although, compared with protein-coding genes, we know less about pseudogenes. The research of pseudogenes is expanding quickly. Therefore, more functional investigations are needed.
Abstract No. 722

**The Effects of Hedgehog Pathway Inhibitors: Vismodegib and Gant61 on Cisplatin-resistance, Pemetrexed-resistance, Vinorelbine-resistance and Non-resistance Non-Small Cell Lung Cancer Cell Lines**

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**Introduction** Resistance to chemotherapy is an intractable problem for the treatment of non-small cell lung cancer (NSCLC), therefore the development of targeted pathway inhibitors would be important and necessary to overcome chemo-resistance and to reduce cancer reoccurrence. Hedgehog pathway is involved in the proliferation and migration of tumor cells. Vismodegib can block the activities of the Hedgehog-ligand cell surface receptors PTCH and/or SMO and suppress Hedgehog signaling. Gant61 is an inhibitor for GLI1 as well as GLI2-induced transcription, and inhibits the DNA binding ability of GLI1.

**Method and Materials** Inhibitors of hedgehog pathway: Vismodegib and Gant61 have been applied in this research project. As a substrate for the whole-cell dehydrogenase activity estimations, 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) was also used in our research project. The amount of formazan product was evaluated after dissolved fully with isopropanol by a spectrophotometer. The half maximal inhibitory concentrations of Vismodegib and Gant61 were calculated according to the absorbance of the formazan solution.

**Results** The half maximal inhibitory concentrations of Vismodegib on HCC827 cells, HCC827 Cisplatin-resistance cells, HCC827 Pemetrexed-resistance cells and HCC827 Vinorelbine-resistance cells were respectively 51μM, 14.7μM, 34.6μM, and 48.4μM; meanwhile the half maximal inhibitory concentrations of Gant61 on the cells were respectively 13.9μM, 15.9μM, 27.7μM, and 22.9μM.

**Conclusion and Outlook** The survival rate of cancer cells decreased with the increase of Vismodegib or Gant61 concentration. Vismodegib or Gnt61 has shown different inhibitory effects on different drug-resistant cells. For the next step, it has been planned that the effective concentration of Vismodegib and Gnt61 on the targeted gene expression of Hedgehog pathway will be investigated on the NSCLC cells.
Abstract No. 723

Response to Checkpoint Inhibition in Lung Cancer with Molecular Driver Alterations

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Introduction: Checkpoint inhibitors can be used as second-line therapy for NSCLC and as first-line treatment for patients with high PDL-1 expression. While there is evidence that patients with EGFR mutations do not respond well to checkpoint inhibition, little is known about the prognostic value of other driver alterations. We present a retrospective analysis of molecular drivers in patients treated with checkpoint inhibitors.

Methods: Patients treated with checkpoint inhibitors were identified and treatment data was extracted from our clinic databank. Next generation sequencing (NGS) was performed using the Oncomine Focus Panel (Life-Technologies), which identifies point mutations, copy number variations and translocations/gene fusions, and results were correlated with treatment response, and progression free survival.

Results: We identified 69 patients treated with checkpoint inhibitors including 17 patients with squamous cell and 39 patients with adenocarcinoma. 44 patients were current/former smokers and 66 received Nivolumab. We found 15 driver alterations: 4 KRAS mutations, 3 PiK3Ca mutations, 2 EGFR mutations (1 Del19 and 1 rare), 1 KIT mutation, 1 RET Fusion, 1 Met mutation, 1 ERBB2 amplification, 1 FGFR1-amplification and 1 MYC amplification. Responses were seen in patients with FGFR-1 amplification, MYC amplification and rare EGFR mutation, and in some patients with KRAS mutation or PiK3Ca mutation. No response was seen in patients with RET, MET, EGFR Del19 or KIT. Mean progression free survival was 38.5 weeks in KRAS positive patients and 22.5 weeks in PiK3Ca positive patients.

Conclusion: Checkpoint inhibition is an effective treatment option for some patients with NSCLC. Patients with KRAS mutation or PiK3Ca mutation seem to respond at least as well as wild-type patients, whereas patients with RET fusion or MET, KIT or EGFR Del19 mutation seem to respond poorly. Further larger cohorts must be studied to determine the best treatment sequences for patients with various genetic driver alterations.
Abstract No. 724

Phosphorylation of R-SMADs in Non-Small-Cell Lung Cancer

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Lung cancer leads to 1.6 million deaths worldwide and belongs to the ten most common causes of death. In case of Non-Small-Cell Lung Cancer (NSCLC) therapy is stage-dependent and only early-discovered tumors can be treated surgically. In advanced stages chemotherapy is the primary choice and radiation is used in addition. Further treatment options are targeted therapies, but these are biomarker dependent and relatively rare. Investigations of the TGFβ signaling pathway in NSCLC tissues revealed activation of Receptor-regulated SMADs (R-SMADs) with a high frequency of C-terminal phosphorylation.

TGFβ signals are involved in processes of tissue homoeostasis, cell differentiation and proliferation. Within the TGFβ pathway small mothers against decapentaplegic (SMAD) proteins are the mediators of the signal response and serve as direct substrates for phosphorylation through the TGFβ-transmembrane-receptor-I (TβR-I). Phosphorylated SMAD molecules can translocate into the nucleus and act as transcription factors. R-SMADs consists of two highly conserved domains (MH1 and MH2), wherein MH2 possess a Serine-motif at the C-terminus for TβR-I phosphorylation. C-terminal SMAD-phosphorylation is associated to cytostatic and antiproliferative cell processes. MH1 and MH2 are connected by a divergent linker region, which possess additional phosphorylation sites for intracellular kinases (e.g. MAPK, JNK). Nevertheless, NSCLC proliferate, therefore we hypothesized that SMAD-linker phosphorylation can be a mechanism to overcome antiproliferative effects from C-terminal signaling.

At this time, no data is available for SMAD linker phosphorylation in NSCLC and other lung diseases. It is also unclear which consequences result from these in lung and tumor cells. Recent studies on various NSCLC cell lines revealed a highly phosphorylated side population of an R-SMAD linker region that could be affected by the inhibition of intracellular, non-TβR kinases. Further results from immunofluorescence microscopy elucidated a close localization to the nucleus exclusively during mitosis.
Abstract No. 725

Cisplatin-Resistant Phenotype: Characterization, adaptation to EGFR signaling and sensitivity to EGFR inhibitors in NSCLC cells

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Abstract: Lung cancer remains the leading cause of cancer deaths worldwide and Platinum plays a central role in the therapy, and the emergence of platinum resistance is a significant obstacle for clinical management of the disease. Resistant tumor cells follow distinct evolutionary paths to survive. Understanding the molecular mechanisms results in novel agents and enhances CDDP sensitivity. This study aims to analyze the impact of acquired cisplatin resistance on EGFR phosphorylation downstream signaling and effectiveness of three generations of EGFR inhibitors.

Experimental design and Methods: An isogenic clinical model of cisplatin resistance was used to induce resistance in a panel of EGFR mutant NSCLC lung cancer cell lines (H838, HCC827, H1975 and H1650 NSCLC cells) and H1339, an SCLC cell line.

Cells treated with cisplatin (1µg/ml/3hrs/week) followed by recovery periods over of 4 weeks. Cisplatin-resistant phenotype (CRP) derived from original, age-matched naïve cells. CRP cells characterized by survival (trypan blue exclusion), proliferation (Cell Titer Blue), colony formation (crystal violet), and apoptosis (Annexin V). The critical nodes of EGFR downstream signaling assessed by PathScan EGFR Signaling Antibody Array and EGFR family receptors expression and their phosphorylation through phosphorylation array. The EGFR TKIs (erlotinib, gefitinib, afatinib, and rociletinib) effects on survival, proliferation, and apoptosis of CRP cells evaluated at clinical concentrations.

Results: Characterization studies demonstrated a decreased proliferative capacity of lung tumor cells in response to cisplatin, increased resistance to cisplatin-induced cell death, and enhanced clonogenic survival ability. CRP displayed changes in EGFR receptor family and their phosphorylation. CRP demonstrated altered signaling nodes of EGFR downstream and expression varied from cell line to cell line compared with their respective naïve cells. The three generations of EGFR inhibitors did not show any significant change in potency on the CRP cells in comparison to their naïve cells.
Abstract No. 726

Mutational signatures of a tobacco carcinogen across different strains of inbred mice reveal novel KRAS-addicted candidate oncogenes

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Background: KRAS mutations are frequent in lung adenocarcinoma (LADC) and other bodily tumors representing 30-40% of known cancer mutations overall. Despite intensive research, KRAS mutations cannot be currently targeted, possibly due to their addiction with other, yet unidentified oncogenes.

Objectives: To identify potential addiction partners of KRAS using mouse models of tobacco chemical-induced LADC.

Methods: We used urethane, a tobacco chemical, to induce KRAS-mutant LADC in different strains of inbred mice and established cell lines thereof (n = 9). Cellular RNA was subjected to next generation sequencing using the IonTorrent platform. Data were analysed for transcript abundance and sequence alignment, respectively, by BioConductor on R* and STAR, TOphAT2, and HISAT2 packages on Linux. InSyBio Suite software was used to build gene networks.

Results: We detected 641 genes significantly (5 fragments/Kb transcript/million mapped reads) differentially expressed in LADC cells compared with primary lung epithelial (PLE) cells, their cells of origin. Among those genes, we found proliferin (Prl2c2-5), stemness factors (Oct3/4 and Klf4), and 182 genes participating in altered coexpression hubs. Among 40,427 different single nucleotide variants (SNV) identified specifically in LADC and not in PLE cells, we identified 45 common urethane-specific protein-altering SNV in 34 different genes, in addition to KRAS that was ubiquitously codon 61-mutant in cell lines. Among these candidate KRAS addiction partners, we pinned genes important in cell cycle, DNA repair, and transcription.

Conclusion: A tobacco carcinogen that causes KRAS mutations, also causes changes in gene expression and another 45 mutations. Amazingly, the same changes occurred together in six different cell lines obtained from different mouse strains. We believe that important partners in crime of mutant KRAS lie among those genes.

Acknowledgements: This work was supported by the European Research Council
Tumor microenvironment and its immune components play a critical role in cancer development, progression, and control. In this study we aim to investigate the role of tumor infiltrating lymphocyte subpopulations in lung cancer progression. We demonstrate that conditioned media (CM) from co-cultures of lymphocytes with adenocarcinoma cells (A549) induces epithelial to mesenchymal transition (EMT) of cancer cells. CM from co-cultures of human-lymphocytes with adenocarcinoma cells induced the loss of the epithelial marker E-Cadherin and an increase of vimentin and N-cadherin on mRNA and protein level. A549 cells also showed acquired spindled shape-like morphological changes and an increased migratory phenotype as revealed by wound-healing assay. Increased levels of cytokines such as, IL-8, IL-16, CCL2 and G-CSF were detected in the co-culture CM. To identify the specific T cell subpopulation responsible for the EMT effect observed in human data, Th subpopulations (Th0, Th1, Th9 and Th17) were generated. CM from each of these subpopulations was collected and its functional effects were assessed on tumor cells. We observed an increase in migration and mesenchymal markers expression in cancer cells stimulated only with Th9-CM and Th17-CM. Furthermore, the stimulation of A549 cells with IL-9 itself resulted in EMT and increased migration. Interestingly, co-injecting tumor cells with Th subpopulations (Th9 or Th17) in mouse lung tumor xenograft/orthotopic models resulted in increased lung tumor growth and metastasis. Additionally, tumor homogenates showed decreased mesenchymal markers and increase epithelial markers and angiogenesis markers. This study reveals that specific T lymphocyte subpopulations are able to induce EMT of lung cancer cells, accompanied with a more migratory phenotype.
Abstract No. 728

Reprogramming of Tumor Associated Macrophages by Modulating Wnt/β-catenin Signalling in Lung Cancer

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Data from clinical and experimental studies suggest that Tumor Associated Macrophages (TAMs) contribute to cancer progression and metastasis. We aim to identify and manipulate TAMs specific signalling pathways, which are responsible for shift of macrophage from pro-tumorigenic (M2 like-TAMs) to anti-tumorigenic (M1 like-TAMs). We established and characterized a novel in vitro model, training macrophages with tumor cell for 3 days (M1 like-TAMs) and 5 days (M2 like-TAMs). These M2 like TAMs showed reduced apoptosis in tumor cells, increased tumor cell migration and proliferation compared to M1 like-TAMs. RNA sequencing of M1 and M2 like-TAMs revealed differential activation of Wnt/β-catenin signalling. M2 like-TAMs showed significant upregulation in Wnt/β-catenin signalling. Immunostaining of β-catenin in TAMs of 70 human lung cancer sections revealed that β-catenin is activated in TAMs. Notably, interfering Wnt/β-catenin signalling in M2 like-TAMs by shRNAs of β-catenin, TNKS1/2 and β-catenin inhibitor resulted in cancer regression by phenotypic and functional switch of M2 like-TAMs to M1 like-TAMs. ChIP-qPCR analysis revealed that β-catenin binds to M2 macrophage gene promoters. Interestingly, bone marrow derived macrophages of β-catenin knockout mice exhibit M1 like-TAMs phenotype. Therefore, to study the role of Wnt/β-catenin signalling in cancer cells/TAMs interplay in vivo, we treated adenocarcinoma xenograft model and metastasis lung tumor model with β-catenin inhibitor. Upon treatment, we observed significant reduction in tumor size and increased accumulation of M1 like-TAMs in tumor microenvironment. Interestingly, inhibition of Wnt/β-catenin signalling in TAMs from ex vivo isolated human and mouse lung tumors showed functional switch of M2 like-TAMs to M1 like-TAMs. Thus, intervention of Wnt/β-catenin signalling in TAMs will permit the development of new cancer therapeutics.
Abstract No. 729

Epigenetic alterations in blood associated with chronic inflammation of the lung as potential biomarkers for lung cancer risk

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Lung cancer accounts for the majority of cancer-related deaths worldwide. Inflammation is a considerable risk factor for cancer development in general. DNA methylation changes in blood lymphocytes are promising as risk biomarkers for cancer and have been studied extensively regarding the main risk factor for lung cancer, smoking. However, acquired aberrant DNA methylation as a result of chronic lung inflammation and its role in lung carcinogenesis remains under-examined. Cystic fibrosis (CF) is a monogenetic disease characterized by massive chronic inflammation of the lung due to mucus obstruction and impaired eradication of infectious agents. Thus, in this study we use CF as a model for chronic lung inflammation to investigate acquired inflammation-related alterations in DNA methylation. Data are available from the European CF Twin and Sibling Study, including Illumina 450K methylation data from peripheral blood samples as well as clinical data comprising disease severity and intra-pair discordance from 22 monozygotic twins with CF. In order to identify inflammation-related differentially methylated positions (DMPs), a search strategy was applied, considering intra-pair discordance and the orientation of differential methylation. Further, to discover inflammation-related DMPs with potential functional relevance for lung cancer risk, we compared our data with publically available 450K methylation data from lung cancer versus normal adjacent tissues provided by The Cancer Genome Atlas. The strongest inflammation-related DMPs as well as those with possible functional impact will be tested in blood samples from the Heidelberg lung cancer case control study with individuals, matched for age, gender, smoking status and pack-years to determine their value as blood-based epigenetic risk biomarkers, using targeted bisulfite next-generation sequencing. The intention is to discover DNA methylation changes, associated with chronic inflammation and lung cancer which may be implemented in lung cancer risk assessment.
Abstract No. 730

MiCEE: a ncRNA-protein complex mediates epigenetic silencing and nucleolus organization

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OBJECTIVE: The majority of the eukaryotic genome is transcribed into non-coding RNAs (ncRNAs), which are important regulators of different biological processes in the cell nucleus as part of the machinery controlling chromatin structure. However, the full extent of ncRNAs function has remained elusive. Here we deciphered the function of the microRNA Mirlet7d as a key regulator of the expression of bi-directionally transcribed genes. RESULTS: We found that nuclear Mirlet7d binds ncRNAs expressed from bi-directionally transcribed genes. The Mirlet7d-ncRNA duplexes are further bound by C1D, which in turn targets the RNA exosome complex and EZH2 to the bi-directionally active loci. The exosome degrades the ncRNAs, whereas EZH2 induces heterochromatin and transcriptional silencing. Moreover, this multicomponent RNA-protein complex, which we called MiCEE, tethers the regulated genes to the perinucleolar region, thereby being required for proper nucleolus organization. CONCLUSIONS: Our study demonstrates that the MiCEE complex mediates epigenetic silencing of bi-directionally expressed genes and global genome organization.
Abstract No. 731

Interleukin-17 and -22 Expression in Non-Small Cell Lung Cancer

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Background:
In non-small cell lung cancer (NSCLC) the TNM staging remains standard for prognostic assessment and therapy decisions. Nevertheless, stage-specific outcomes vary significantly, indicating a need for additional prognosticators. In this study we assess the role of interleukin-22 (IL-22), as IL-22 is known to be involved in various lung diseases and to be elevated in lung cancer. Moreover, we evaluated interleukin-17 (IL-17), which depicts a proinflammatory cytokine, but seems to play a dual role in antitumor immunity. We asked the question of their link to prognosis, therapy response and recurrence.

Methods:
Tissue microarrays (TMAs) were generated from formalin-fixed paraffin embedded tissue of curatively resected patients with stage IA-IV NSCLC. TMAs included each 3 cores from the tumor center (CT) and invasive margin (IM). IL-22 and IL-17 expression of the tissue was analyzed by immunohistochemistry via cytoplasmic staining (double-staining: IL-22, CD3 resp. IL-17, CD3).

Results:
IL-22 expression in adeno and SCC does not seem to have a prognostic impact on survival, also regardless of the localization. However, a high IL-22 CT/IM ratio in adenocarcinoma is clearly linked to longer overall survival, this cannot be seen in SCC. For IL-17 a distinct tendency is visible for adeno and SCC in the IM as well as in the CT – interestingly this is more clear for SCC. In SCC a high CT/IM ratio for IL-17 seems to have a positive prognostic impact on overall survival. Patient numbers will be enlarged to validate these findings.

Conclusion:
The cytokines IL-22 and IL-17 seem, depending on histology and location, to have prognostic impact on overall survival in NSCLC. In previous experiments we could show that multispectral assessment of CD8 and PD-L1 clearly correlates with clinical outcome. Adding cytokines to this method might open novel avenues for predicting clinical outcome and therapeutic efficacy.
Abstract No. 732

The dynamics of tumor-stroma interactions in lung cancer

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Lung cancer is the leading cause of cancer-related deaths worldwide due to early metastatic spread and quickly acquired resistance to therapies. These processes can be critically influenced by cancer-associated fibroblasts and extracellular matrix (ECM). They were reported to enhance resistance to multiple drugs and increase tumorigenicity in a xenograft mouse model. However, an unbiased analysis of the dynamic changes in factors secreted upon tumor-stroma interactions was missing.

In this study, we characterize the communication between cancer cells and fibroblasts in the context of ECM to gain insights how these interactions could drive tumor progression. We developed an in vitro model for lung ECM deposition by lung fibroblasts cell line HFL1 and populate the produced ECM with lung adenocarcinoma cell line H1975 and HFL1 fibroblasts. Mass spectrometric proteomic analysis combined with a newly developed cell-specific proteome labelling method allows us to distinguish the cell-of-origin of proteins in the supernatant.

We show that the ECM produced by fibroblasts upon stimulation with Transforming Growth Factor β differs greatly from the ECM produced by unstimulated cells. These two matrixes can greatly influence the levels of multiple proteins in the supernatants and cell lysates. Cocultures of cancer cells and fibroblasts alter the secretion of numerous factors including proteins related to tumor progression such as Hepatocyte Growth Factor and Interleukin 6.

These results suggest the existence of multiple feedback loops between cancer cells and fibroblasts. Such bidirectional interactions may further increase fibroblast differentiation and induce EMT in tumor cells that would facilitate tumor spread and therapy resistance.
Abstract No. 733

Macrophage and cancer cell cross–talk regulated microRNA 147b plays a crucial role in lung cancer progression

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In the lung cancer microenvironment, macrophages are a major component and one of the key effector cells regarding lung cancer development and progression. Cancer cells regulate several miRNAs in immune cells to limit their antitumor response and reprogram them to promote tumorigenesis. The molecular cues that control macrophage/ tumor crosstalk in the lung are only partially understood. Using a microRNA microarray- based approach we have found that miR-147b is highly induced in cancer cells co-cultured with macrophages. We could confirm the upregulation of miR-147b data in different human lung cancer cell lines co-cultured with macrophages as well as human lung cancer tissue. In vivo we could show that after macrophage depletion using the MaFIA-mouse model the expression of miR-147b is significantly downregulated, indicating a macrophage - dependent expression.

Overexpression of miR-147b in A549 adenocarcinoma cells reveals a significant increase in proliferation, colony formation, and migration and resulted in epithelial to mesenchymal transition in vitro. To identify miR-147 target genes especially related to cancer pathways we used the NanoString approach. We found that most of the genes affected by overexpression of miR-147b are related to the MAPK, RAS, and PI3K pathways. To identify target genes regulated by miR-147b, we searched the database microRNA.org. Further, we confirmed selected target genes (FGF14, DTX4 and DUSP8) by luciferase gene reporter assays in miR-147b overexpressing cells. We conclude that interference of this microRNA and further investigations on miR-147b target genes and related pathways will provide new opportunities for lung cancer targeted therapies.
Abstract No. 734

Differences in access to therapy and survival between lung cancer patients treated in hospitals with high and low patient volume

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This observational claims data-based analysis aimed to investigate differences in access to therapy and survival between hospitals with a high vs. low patient volume, in light of the German National Cancer Plan which certifies hospitals as lung cancer centers with at least 200 primary cases a year.

We analyzed 14,113 individuals with incident lung cancer in 2013 who received tumor-directed therapy from health insurance claims covering around 30% of Germany. We grouped hospitals into those with high (HVH) and low patient volume (LVH) depending on number of primary lung cancer cases. HVH were hospitals with >66 primary cases in one year. We used logistic regression models to assess differences in the type of therapy (resection, chemotherapy and radiotherapy), palliative care and whether a DNA mutation analysis of the tumor was performed. 1-year survival was modeled via Cox regression. We adjusted all regression models for age, gender, individual Charlson comorbidities, metastases location and district type of residence.

We found that patients in HVH were more likely to receive a lung cancer resection (OR=2.1, p-value<0.0001), also if a lobectomy was performed it was more likely to be thoracoscopic (OR=1.1, p-value=0.01). Systemic therapy was less common in patients of HVH (OR=0.57, p-value<0.0001). If treated in a HVH, patients were more likely to receive palliative care (OR=1.2, p-value<0.0001) regardless if it was inpatient (OR=1.2, p-value=0.01) or outpatient (OR=1.3, p-value<0.0001). We did not find differences in the likelihood of receiving radiotherapy or a mutation test. Patients in HVH were more likely to still be alive after 2 years (HR=0.8, p-value=<0.0001).

Lung cancer treatment differs to some extent between HVH and LVH. Possibly curative lung resections were more likely in HVH and more often performed as the less invasive thoroscopic approach. In addition, access to palliative care was better for patients treated in HVH.
Abstract No. 735

Role of p73 alterations for the malignant neuroendocrine SCLC phenotype

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Lung cancer is the leading cause of cancer-associated mortality world-wide with non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) being the most common subtypes. Although newly diagnosed SCLC is exquisitely sensitive to first-line chemotherapy, the disease eventually progresses rapidly in virtually all patients resulting in a 5-year survival of less than 10%. There is therefore an urgent need to develop novel therapeutic strategies. Unfortunately, SCLC are genetically driven by inactivating mutations in the tumor suppressor genes p53 and RB1 and not by druggable oncogenic drivers.

Using targeted genome sequencing of SCLC patients, we identified cases with structural rearrangements in the p73 transcription factor gene that delete the exons encoding the N-terminal p73 transactivation domain and cause production of N-terminally truncated ΔNp73 proteins. More frequently, in approximately half of all SCLC cell lines and primary tumor samples, we observed loss of DNA methylation in an intronic CpG island of the p73 gene, which thereby gains promoter activity and also drives expression of N-terminally truncated ΔNp73 proteins missing the transactivation domain. In both cases the resulting transactivation-deficient ΔNp73 proteins sequester and inactivate any tumor suppressive full-length p73 proteins.

These genetic and epigenetic p73 alterations are found to be mutually exclusive with NOTCH1-4 receptor mutations. Mutually exclusive genomic events provide strong genetic evidence that the altered genes are functionally linked in a common biological pathway. As NOTCH signaling is functioning as a repressor of neuroendocrine differentiation during lung development, this links p73 alterations to the neuroendocrine phenotype of SCLC and suggests that p73 in interplay with NOTCH might play a role to drive and maintain the malignant NE SCLC phenotype.
Abstract No. 736

Regulation of Glycodelin Expression – an Immunomodulatory and Pregnancy associated Protein in NSCLC

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Background: Glycodelin is a protein first described as an immune system modulator during the establishment of pregnancy. Later, it was found to be overexpressed in non-small cell lung cancer (NSCLC). This and the fact that glycodelin serum levels correlated with treatment response, suggests it as a follow-up biomarker for NSCLC patients. Signaling pathways involved in the regulation of glycodelin expression in NSCLC are unknown. Therefore, the aim of this study was to advance our knowledge about these pathways in NSCLC.

Methods: A lung adenocarcinoma (H1975) as well as a lung squamous cell carcinoma (2106T) cell line were treated with different pathway inducers (lysophosphatidic acid (LPA), phorbol 12-myristate 13 acetate (PMA), epidermal growth factor (EGF), heparin-binding EGF-like growth factor (HB-EGF), transforming growth factor-β (TGF-β1, -2)) and analyzed regarding glycodelin expression. Downstream of those inducers are the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)-, rapidly accelerated fibrosarcoma (RAF)/extracellular signal-regulated protein kinase (ERK)- and protein kinase C (PKC)-pathway, which were also examined. Furthermore, these investigations included the transcription factors junB proto-oncogene (JUNB), activator of transcription 3 (STAT3) and nuclear factor κB1 (NFκB1). Glycodelin mRNA expression was determined by qPCR, while the protein was detected by immunoblot analysis.

Results: In NSCLC cells, the pathway inducers LPA, PMA, EGF and HB-EGF stimulated glycodelin expression by activating downstream PKC. TGF-β1 and -2 induced glycodelin expression without affecting PKC. The downstream pathway including AKT repressed glycodelin expression. Contradictory effects could be demonstrated for the RAF/ERK-pathway and for the transcription factors JUNB, STAT3 and NFκB1.

Conclusion: These results revealed that some signaling pathways, targeted by NSCLC drugs, also influenced glycodelin expression and therefore might have an impact on immunomodulation mediated by cancer cells. Additionally, the identification of glycodelin expression regulating candidates in NSCLC might give us hints for potential strategies to weaken the immune system defense of lung tumors.
Abstract No. 737

**Sequential biopsies and blood sampling from patients with inoperable NSCLC – 5 years of experience with TLRC cohort assembly.**

Sabine Wessels¹, Marc A. Schneider¹,* Ingrid Heinzmann-Groth¹, Magdalena Stephan¹, Karin Schnorr-Teichert¹, Jonas Kuon⁶, Ralf Eberhardt⁷, Arne Warth⁷, Simone Hummler⁷, Claus Peter Heussel¹, Felix JF Herth¹, Michael Meister¹, Thomas Muley¹, and Michael Thomas¹

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Targeted therapies and immune therapy meanwhile are of high importance for non-small cell lung cancer treatment. For diagnosis in late stages, biopsies were routinely obtained. Since therapy resistance is often accompanied by molecular changes of the tumor, re-biopsies are essential for elucidating further treatment options. Primary and re-biopsies therefore offer the possibility for research projects to investigate molecular mechanisms associated with therapy resistance and outcome. Here, we describe our experiences after 5 years of TLRC cohort assembly.

Study nurses of the Lung Biobank Heidelberg (LBBH), member of the DZL Platform Biobanking, initially screened patients for inclusion criteria, followed by obtaining informed consent. Snap-frozen biopsies, blood samples and patient data were collected at time of diagnosis, during therapy and, if feasible, at time of progression. Samples were stored in the LBBH and partly processed for research projects.

In total, 455 patients were included for sequential biomaterial acquisition in the past 5 years. 241 primary snap-frozen biopsies were collected with 186 (77.1 %) matching all primary inclusion criteria. For 21.5 % of these patients (n=40), a re-biopsy was taken. Processing and characterization of the cryo-samples exhibited that the median tumor content of the biopsies was 35.4 %. Accompanying blood samples were obtained from each patient with a median of 6.9 (range 1-42) follow-up samples.

Our experiences show a large number of dropouts of patients until time of re-biopsy not only because of patient-derived, but also of sample specific factors. Blood samples are of increasing importance especially for so-called liquid biopsies. However, obtaining enough circulating tumor cells/cell free tumor DNA as well as sensitivity and specificity of molecular analyses remain a significant problem for most cancer types. Therefore, the classical tissue biopsy remains the gold standard for molecular research such as mutation analysis, epigenetic studies, nucleic acid expression profiles or immunohistochemistry.
Abstract No. 738

Re-education of tumor-associated macrophages by modulating histone deacetylases in lung cancer

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Background: Lung cancer is one of the most common cancers in terms of incidence and mortality around the world. Tumor microenvironment plays pivotal role in progression and metastasis of most cancer types. Within the established tumor microenvironment, tumor-associated macrophages (TAMs) are one of the most abundant stromal cell types. M1 macrophages possess anti-tumor capability and M2 macrophages manifest pro-tumor feature.

Objectives: This study investigated epigenetic mechanisms of macrophages polarization and re-education of TAMs by modulating histone deacetylases (HDACs).

Methods: Human naive macrophages (M0) were generated from PBMC and were polarized to M1 or M2 macrophages that were either used for cell culture or animal experiments. Additionally, human TAMs were isolated from human lung cancer tissues.

Results: Both in vivo and in vitro, M2 macrophages led to increased tumor cell proliferation, migration and decreased apoptosis. Of note, RNA-Seq analysis showed that HDAC2, which belongs to class I HDAC family, as one of the significantly upregulated genes in M2 macrophages. Furthermore, upregulation of HDAC2 at protein level and significantly elevated HDAC activity was observed in M2 macrophages. Similar regulation of HDAC2 was found in M2-like TAMs. Interestingly, suppression of HDAC2 employing pharmacological (HDAC inhibitors; SAHA, VPA) or genetic (HDAC2-siRNA) approaches in human- and mouse- bone marrow-derived M2 macrophages and as well as in isolated TAMs from human- and mouse-lung tumors, led to upregulation of M1 markers (TNFα, CCR7, IL12) and downregulation of M2 markers (IL10, ALOX15, CD206). Notably, repolarization of M2-like TAMs reversed the tumor cells functions (proliferation, migration, and apoptosis). In addition, RNA-seq from HDAC2-knockdown M2 macrophages lead to identification of target genes that are involved in macrophage repolarization processes.

Conclusions: Suppression of HDAC2 switches M2-like TAMs into an M1-like phenotype and regulates tumor cell functions. Modulation of HDAC2 may provide a novel strategy for TAM repolarization and cancer therapy.
Biobanking and Data Management Platform: Hands-On Session
Hands-on – Datawarehouse solutions in the DZL

February 8, 2018 | 14:10 – 15:40 | Room Sprudelhof

The DZL data management team offers a hands-on experience for any person interested in possibilities and benefits of datawarehouse solutions in the DZL. Besides a permanent info point, where users can make their own experiences with our systems, there will be a tutorial session which will take place after poster viewing.

Title 1: The DZL central datawarehouse (i2b2)

Presenter: Raphael Majeed, Mark Stöhr & the data management team

Description: The DZL Datawarehouse is developed to allow consortium-wide queries on a large data pool. It enables researchers to find patient sets fitting certain criteria, e.g. for retrospective research or prospectively for patient recruitment. Currently, the datawarehouse contains data from 18 registries/cohorts/databases and biobanks submitted by the sites at Großhansdorf, Hannover, Heidelberg, Gießen and Munich. More data and features will be made available on a continuous basis in the next months. During the hands-on session, the datamanagement team will present functions of the datawarehouse platform i2b2. You will learn the following:

- How to perform queries in i2b2 web interface
- What information is available through i2b2 (number of patients fitting criteria, breakdowns,…)
- How to participate in the development of the DZL ontology

The DZL Datawarehouse can be accessed via: https://data.dzl.de/webclient/, with the login name and password provided to every DZL PI by an email from the data management team.

The Collaborative Metadata Repository CoMetaR provides information (descriptions, corresponding nomenclature codes, …) about clinical parameters (common dataset), phenotypes and specimen and is available at: https://data.dzl.de/cometar/web/

Title 2: TLRC site specific report on data management

Presenter: Karsten Senghas & the TLRC data management group

Description: The members of the TLRC data management group report on the ongoing data integration efforts and the process to establish tranSMART locally as a clinical DWH. The aim of our initiative at the Thoraxklinik Heidelberg is to integrate heterogeneous data sets in a single structurally integrated form available for clinical research. tranSMART as a software shares similarities with i2b2 and its data schema, but differs in functionality and focus. Everybody interested is invited to discuss and share their experience on the following topics:

- Similarities and differences between tranSMART and i2b2
- Suitability of analytic functions in tranSMART for clinical research
- Challenges in implementing tranSMART for operative use
Biobanking and Data Management Platform: Abstract No. 801 – 809
Abstract No. 801

Introducing DIOP: the DZL Integrative Omics Platform

All DIOP members and participants of the kick-off meeting: \(^1\) Robert Bals\(^2\), Marek Bartkuhn\(^3\), Sebastian Boutin\(^4\), Alexander Dalpke\(^4\), David DeLuca\(^5\), Iven Fellhauer\(^4\), Jan Fuge\(^5\), Karoline Gaede\(^6\), Goesmann Alexander\(^3\), Torsten Goldmann\(^6\), Andreas Günther\(^3\), Christian Kalberlah\(^3\), Ursula Klingmüller\(^4\), Inke König\(^6\), Mario Looso\(^3\), Raphael Majeed\(^3\), Thomas Muley\(^4\), Oliver Rupp\(^3\), Clemens Ruppert\(^3,\*\), Matthias Schlesner\(^4\), Karsten Senghas\(^4\), Mark Stöhr\(^3\), Burkhard Tümmler\(^5\), Jasmin Wagner\(^3\), and Jochen Wilhelm\(^3\)

\(^1\) DIOP
\(^2\) Cosyconet, Homburg/Saar
\(^3\) Universities of Giessen and Marburg Lung Center (UGMLC)
\(^4\) Translational Lung Research Centre Heidelberg (TLRC-H)
\(^5\) Biomedical Research in Endstage and Obstructive Lung Disease Hannover (BREATH)
\(^6\) Airway Research Centre North (ARCN)
\(^\*\) Presenting author

The infrastructure of the DZL-data warehouse (DZL-DWH) is developed using a dynamic development paradigm, hence allowing continuous improvement. The first patient cohorts and clinical databases, as well as biobanks are already connected and data sets are being uploaded. Besides linking biomaterials, phenotyping and imaging data, there is a need to make –omics-data available for lung research and to establish a reliable and effective approach for the integration into the central DZL-DWH. Management of large data sets, processing and analysis represent major hurdles in this context. This is even more challenging, if several sets of –omics data are produced by various platforms and in different formats. Likewise data protection procedures have to be adopted and updated. To coordinate all existing expertise within the DZL in the fields of basic, translational and clinical lung research, IT and software development, bio-informatics and –mathematics and systems biology, a DZL integrative Omics Platform (DIOP) has recently been established. A kick-off meeting with experts in this field from all DZL-sites took place on November 11\(^{\text{th}}\) 2017. The goals and work packages of the DIOP were defined and will be presented.
Abstract No. 802

**Data Management at the Comprehensive Pneumology Centre, Munich (CPC)**

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Enabling high-end translational research approaches, the CPC-BioArchive oversees a large variety of biological samples from patients with both rare as well as highly prevalent lung diseases. Their efficient and secure management with modern data protection standards requires complex, yet user-friendly, data management on all levels from daily supervision of sample flow to the generation of large data repositories. To achieve this, data at the CPC-BioArchive is managed on three levels:

- The first clinical registers have been imported into a clinical database, which allows in-hospital, personalized oversight of patients and their associated data. To implement its use into clinical routine, user-friendly tools were created, which allow the overview of individual disease courses as well as longitudinal approaches in entire patient cohorts. Dedicated front-end solutions are developed to allow highly flexible data entry by clinical partners while maintaining accepted database standards. Patient data are pseudonymized by an external identity management provider before, subject to their consent, biological samples are provided to the CPC-BioArchive.

- On the sample management level, a customized commercial, web-based software package allows day-to-day routine while connecting 6 different departments at 3 different sites and two hospitals. The newly incorporated infrastructural information allows real-time sample tracking from database entry over storage to its issue to a particular researcher and project.

- Large, project associated datasets including OMIC data and clinical as well as imaging datasets (bench-to-bedside), are united in a data warehouse, access to which follows a highly regulated role-concept. The latter differentiates between initial data research at a project’s concept stage and result-driven, full data retrieval.

The system outlined above allows efficient, yet regulated sample management. With the steadily growing number of samples, we envisage the increased automation of their processing and storage. In addition, further large datasets (e.g. from extensive proteomics approaches) will be integrated into the data warehouse.
Abstract No. 803

**DZL Platform Biobanking: Biobank Profiles**

*Clemens Ruppert*¹, Robert Bals², Karoline Gaede³, Anne Hilgendorf⁴, Thomas Illig⁵, Ina Koch⁶, Michael Lindner⁶, Petra I. Pfefferle¹, Thomas Muley⁶, Andreas Günther¹, and all members of Platform Biobanking and Data Management⁷

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⁷ DZL-Platform Biobanking

*Presenting author*

Biomaterials are valuable tools in translational lung research. In order to provide research projects with biomaterials, currently 8 Biobanks from 6 DZL-sites are connected in the platform Biobanking and develop and harmonize standards and procedures regarding collection, processing and preservation of biomaterials. The samples itself are decentrally stored, and each local biobank has a different focus with regard to the collected phenotypes and specimen. The BioMaterialBank-North including Research+Center Borstel, LungClinic-Grosshansdorf and UKSH-Lübeck, mainly collects blood, sputum and lavage samples from asthma, allergy, COPD, lung cancer, TB, sarcoidosis and other DPLDs. A specific feature is the use of the HOPE fixation technique and the isolation of primary cells. Hannover runs a central Biobank (HUB) for the Hannover medical School. Samples are stored in LN2-tanks and an automated -80°C repository (BiOS Hamilton). At UGMLC two biobanks exist. The Comprehensive Biomaterial Bank Marburg (CBBM) is the central biobank of the medical faculty and hosts lung tumor tissue and liquid samples from KIRA and ERA cohorts. The UGMLC-Giessen-Biobank has a strong focus on DPLD, PH, COPD and pneumonia. Next to liquid samples, it comprises a large collection of explanted lungs and isolated primary cells. The biobank is connected to large patient registries including the European-IPF-registry and the Giessen-PH-registry. The COSYCONET-biomaterialbank collects liquid biomaterials from recruited COPD patients in a long-term follow up. The LungenBioBank-Heidelberg (LBBH) is located at the Thoraxclinic and embedded in different network structures (NCT-tissue-bank, BioMaterialBank-Heidelberg). The major expertise is on lung cancer (FFPE, native tissue, cells, liquids) and LC-data management. In Munic biobanking is established at two sites, the CPC-M Biomaterial Archive and the Asklepios Biobank for Lung Diseases at Gauting. Tissue, BALF, sputum, tracheal aspirates, blood derivatives are collected mainly from interstitial and chronic obstructive lung diseases, asthma. A special feature is primary cell extraction, immune monitoring and PCLS-culture.
Abstract No. 804

Establishment of patient-derived primary Non-small cell lung cancer (NSCLC) cell cultures using the DZL-SOP 34

Marc A. Schneider¹,*, Christa Stolp¹, Martin Fallenbuechel¹, Elisabeth C. Xu¹, Carmen Hoppstock¹, Arne Warth², Felix Lasitschka², Hendrik Dienemann¹, Hauke Winter³, Thomas Muley¹, and Michael Meister¹

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For basic research, the use of cancer cell lines is indispensable. Most commercial permanent cell lines are very old and cultivated for several dozen passages. Chromosomal changes and differences of cellular expression profiles may be a consequence, so that these cell lines probably highly differ from the original tumor.

In the DZL-wide SOP 34, we describe the processes of establishing patient-derived primary cell cultures from NSCLC tumors. Tumors are processed (digested or minced, filtered and centrifuged) within half a day. The single cell suspension is then cultured adherent with or without feeder-cells or on agarose coated flasks.

We established more than 70 cultures from adeno- and squamous cell carcinoma of the lung. More than 30 cultures grew as spheroids. Besides the tumor cultures, we also generated cultures from matched non-neoplastic lung tissues with this SOP.

With the DZL-SOP 34, it is possible to generate patient-derived primary cell cultures from NSCLC tissues which can be used for DZL-wide research purpose. The establishment of primary cell cultures can increase the quality of investigations, since these cultures may better reflect the patient status compared to permanent cell lines.
Abstract No. 805

Establishment of patient-derived bronchial air-liquid interface (ALI) cultures using the DZL-SOP 36

Marc A. Schneider1,*, Nicolas C. Kahn1, Christa Stolp1, Martin Fallenbuechel1, Elisabeth C. Xu1, Carmen Hoppstock1, Arne Warth2, Felix Lasitschka2, Michael Kreuter1, Felix JF Herth1, Hendrik Dienemann1, Hauke Winter1, Thomas Muley1, and Michael Meister1

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The bronchial epithelium is involved in various processes as mucus clearance or the control of inflammatory reactions. The airway epithelium consists of a three dimensional cell layer, which is established during a specific cell differentiation. Therefore, 2D cell cultures are only conditionally suitable for in vitro research. Cell culture models that mimic the in vivo situation can be a useful tool to investigate the functions of the bronchial epithelium.

Using the DZL wide SOP 36, we describe the processes of creating patient-derived ALI cultures. Initially, bronchial epithelial cells can be obtained from surgical specimens or bronchoscopic sampling. After processing the cells, the single cell suspension is expanded in 2D and transferred to an air-liquid interface after 3-7 days. Around three weeks later, the functional ALI cultures are established.

Differentiated, functional ALI cultures could be established using surgical material as well as bronchial cells received, in our case, by bronchoscopic microsampling. The cultured cells expressed bronchial epithelial markers such as mucin 5, uteroglobin (also known as CC10), keratin 5 and tubulin b IV. Moreover, a high frequent beating of the cilia could be observed under the microscope.

This SOP enables a generation of patient-derived bronchial ALI cultures that can be used for DZL-wide research purpose. This will facilitate the investigations and the understanding of bronchial epithelium functions in ex-vivo models.
Abstract No. 806

Establishing tranSMART in a clinical context to support data exploration and data exchange across research platforms

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Background: Clinical IT-systems store large amounts of heterogeneous and structurally divergent patient data to support clinical routines. In this context generated medical findings are suitable for secondary use when annotated with metadata. Unstructured and structured clinical data sets, such as letters, reports, and text files together with our clinical registries (e.g. tumor documentation) embedded in the hospital information system (HIS) represent a valuable source for phenotype data. The aim of our initiative is to establish tranSMART at the Thoraxklinik Heidelberg to integrate heterogeneous data sets in a single structurally integrated form.

Methods: Domain experts have selected relevant parameters and provided metadata for our in house research activities and interoperability with the German Center for Lung Research (DZL) as well as other Health Research Centers (DZGs). Data primarily recorded by physicians in daily routine or by dedicated study nurses in clinical trials are extracted by standard queries from the SAP ISH-MED based HIS. Talend Open Studio is then used to upload the data into tranSMART and basic parameter validation is executed against the metadata definitions.

Results: tranSMART is deployed within the clinical network through VMware virtualization technology and available for selecting patient cohorts of interest. Basic statistical and graphical features visualized enable data exploration. In addition, some more advanced statistical analyses might be done e.g. survival analyses.

Conclusion: tranSMART is helpful to store heterogeneous data in an integrated form usable for the generation of new research hypotheses. Using our in house developed Generic Case Extractor (GCE) software specific cohorts and data sets can be extracted for exchange or further analyses with data mining tools or advanced statistical software (R, SPSS). The proof of principle for our approach could be demonstrated in a pilot project. We are now focused to bring our installation to operative use.
Abstract No. 807

CoMetaR – a Collaborative Metadata Repository for Biomedical Research Networks

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Introduction:
In the DZL, there are many different local electronic data capture systems in use, e.g. secuTrial, Filemaker etc. This circumstance hinders researchers from performing consortium-wide queries quickly and with least effort. Therefore, a central data warehouse (i2b2) is used to which every site uploads their data. Semantic integration of lung research data requires multiple existing terminologies like LOINC and SNOMED-CT, in addition to custom lung research specific concepts. Aim of this project is to realize a platform that visualizes the DZL metadata ontology and enables medical documentalists and researchers from all participating institutions to take part in the development process.

Methods:
Our requirement analysis resulted in the following statements: The ontology has to be visualized and searchable through an easily accessible user interface. Medical documentalists need to maintain the ontology, which should be possible without additional software. The description format has to be extendable. Any DZL member should be able to contribute expertise. Trained specialists from anywhere should be able to take part in editing the ontology independently and simultaneously. Changes to the ontology need verification.

Results:
We developed a platform for independently maintaining an ontology from different sites combined with a web interface for visualization. All metadata concepts can be explored through a web browser. For a selected concept the user is offered further details. RDF is designed for ontology representation, it is extensible and has various representation formats. Concept hierarchy is realized through Simple Knowledge Organization System (SKOS). All RDF files are stored in a GIT repository. Transmissions are verified through consistency checks. Afterwards, the updated ontology is immediately loaded into the i2b2 server.

Discussion:
CoMetaR may be used for arbitrary SKOS ontologies. It is especially suitable for management of biomedical and other evolving ontologies. Additional functionality may be integrated into the web interface through javascript extensions.
Abstract No. 808

**Multidimensional Classification of Lung Disease Specific Parameters in Research Networks**

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**Introduction:**
For biobanking, there are two essential information fields: the patient's phenotype and specimens. The DZL-wide used vocabulary for those items was a flat historical grown list. Researchers complained about missing items, missing specification of samples and the lack of a proper hierarchical structure. Extending the existing list with all relevant combinations of samples and specifications would neither be manageable nor maintainable.

**Methods:**
The 27 members of the DZL data management and biomedical research team analysed the requirements for a new classifications system before the data management team developed a solution: (1) We need dynamically recombinable parameters and specifications instead of a rigid flat list. (2) We must describe the items' natural hierarchical order. (3) The new list has to be complete and contain all characteristics used by any study site. (4) The list has to be extendable. (5) For effective communication, we need to assign codes.

**Results:**
We developed a classification system complying with all requirements: (1) Phenotypes range in only one hierarchical tree, samples are divided into seven categories, each of them including their own appropriate detail dimensions like "derivative", "extraction" and "fixation". (2) Each dimension itself is ordered hierarchically. (3) The resulting ontology includes 215 phenotypes (before: 115), 115 sample types and 37 sample specifications resulting in 3718 combinations (before: 68 partly specified samples). (4) The classification system can be extended by adding dimensions or adding items to existing dimensions. (5) Each item composition has an unique code.

**Discussion:**
The system is integrated in our central datawarehouse, offering more meaningful information than a flat list. E.g. when querying for the number of patients with "blood plasma" samples, results will also include patients with "citrated plasma" samples. Corresponding codes from other terminologies like SPREC, LOINC, SNOMED-CT or ICD-10 were annotated where available.
Abstract No. 809

Data Integration in the DZL Central Data Warehouse

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To support consortium-wide retrospective research as well as prospective patient recruitment, we integrate data from all participating DZL study registers, biobanks and databases into our central data warehouse (i2b2). The graphical web interface is designed to allow users without technical background to formulate meaningful queries. The underlying data pool includes copies of phenotypical/clinical data and information about existence of bio-samples and imaging data. The original data and biomaterial stays at the study site. Patient-identifying data will only be used for pseudomization and is not included in the data transfer process.

The integration process involves these consecutive steps: (1) Choose fields for transfer. (2) Map columns / metadata to the DZL ontology. (3) Write the import configuration. (4) Export all data from the local database system. (5) Run our import software. Steps 1-3 are a one time effort. Step 2 and 3 are a service of the central data management team, but still require a dialog with the local data managers as well as subject experts to ensure correct mappings. Steps 4 and 5 are repeated periodically to keep the database up-to-date. It is important to identify the patients who signed the DZL Broad Informed Consent (BIC) or at least a compatible consent with regard to data transfer. Our software is a Java program which is executed through one simple click.

The DZL ontology / metadata catalogue includes 692 hierarchically structured concepts (e.g. diagnoses, smoking status, lung function). End of November 2017, out of the 68 reported study registers, we integrated data from 18 registers and 9 registers refused data transfer due to reasons like missing patient consent.

Future work will include further register integration and development of technical infrastructure allowing automated requests for bio-sample, imaging and omics data.

Detailed DZL ontology: https://data.dzl.de/cometar/web/
i2b2 query interface: https://data.dzl.de/webclient/
Abstract No. 901

Validation of Fourier Decomposition derived pulmonary blood flow using dynamic contrast enhanced MRI in COPD patients

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Introduction: Fourier Decomposition (FD) MRI [1] can be used for simultaneous perfusion and ventilation assessment of the lung. Although a quantification method from arterial spin labeling was applied to FD perfusion imaging [2], no study validated this quantification method in a patient cohort with dynamic contrast enhanced (DCE) MRI. Therefore, in this study a comparison of pulmonary blood flow (PBFFD) derived by FD and PBFDCE derived by DCE is conducted in chronic obstructive pulmonary disease (COPD) patients.

Methods: FD and DCE data was acquired for 20 COPD patients. FD post-processing of one coronal acquisition was carried out using the PREFUL method [3] and quantified according to [2]. PBFDCE was calculated as described elsewhere [4]. The DCE slices were averaged to match the slice thickness of the FD acquisition. Excluding large vessels, the lung parenchyma was segmented as region of interest (ROI), and further divided in left and right and also upper and lower left/right sub-ROIs. Median PBFDCE and PBFFD was computed for the whole lung and all ROIs. Finally, the correlation coefficient r for PBFFD and PBFDCE was determined.

Results: High correlation was obtained for the whole lung (r=0.74, p<0.01), the right lung (r=0.72, p<0.01) and the left upper sub-ROI (r=0.86, p<0.01). Moderate correlation was found for the left lung (r=0.7, p<0.01), the upper right (r=0.54, p=0.01) and the lower right sub-ROI (r=0.67, p<0.01). The low correlation coefficient of the lower left sub-ROI (r=0.37, p=0.1) could be explained by heart-movement, affecting FD analysis.

The median PBFFD of all patients was significantly higher than PBFDCE (PBFFD,median=69.8 [Q1:47.3-Q3:86.2] ml/min/100ml, PBFDCE,median=23.5 [Q1:19.5-Q3:45.2] ml/min/100ml, Wilcoxon signed-rank test: p<0.01).

Conclusion: A high correlation of DCE and FD perfusion measurements is demonstrated, but also problems likely related to cardiac motion in the left lower lung.

References:

Abstract No. 902

**Repeatability of quantitative lung ventilation imaging by magnetic resonance (MR) fluorinated (19F) gas washout dynamics in free breathing**

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**Introduction:** ¹⁹F pulmonary MR gas tracers are a promising alternative to hyperpolarized gases to quantify regional lung ventilation since it can be used at thermal polarization and can be inhaled over several breaths in a normoxic gas mixture. Recently, dynamic ¹⁹F gas washout MRI in free breathing has shown an excellent correlation to forced expiratory volume in 1 second which is the clinical standard to evaluate the severity of COPD. However, for application in longitudinal patient studies knowledge of its repeatability is of high interest. The purpose of this study was to investigate the repeatability between two scans of regional lung ventilation quantification using ¹⁹F gas washout MRI in free breathing in healthy volunteers.

**Methods:** Eight healthy non-smokers were examined twice within one week with both ¹⁹F gas MRI and lung function test. ¹⁹F gas washout MRI was performed in free breathing after inhalation of 30L of a normoxic gas mixture of perfluoropropane and then switching to pure oxygen. Regional ¹⁹F gas washout was quantified by gas washout time ($t_{\text{washout}}$), number of breaths ($n_{\text{breaths}}$) and by fractional ventilation (FV). Repeatability of repeated scans of $t_{\text{washout}}$, $n_{\text{breaths}}$ and FV averaged over the whole lung volume was analyzed with Bland-Altman analysis and by calculating coefficient of variation (COV) among all subjects.

**Results:** For $t_{\text{washout}}$, $n_{\text{breaths}}$ and FV a mean difference and standard deviation of -2.8±10.4.s, -0.02±0.81 and -0.2±1.3% was found with lowest COV for FV (4.4%), followed by $n_{\text{breaths}}$ (8.7%) and $t_{\text{washout}}$ (24.3%).

**Discussion:** While a good repeatability was found for $n_{\text{breaths}}$ and FV, $t_{\text{washout}}$ showed a high variability. Since even in free breathing respiration varies with regard to tidal volume and respiratory frequency, the notably higher variability of $t_{\text{washout}}$ may be explained by its dependence on both tidal volume and respiratory frequency whereas $n_{\text{breaths}}$ and FV only depend on tidal volume.
Abstract No. 903

Fluorescence microscopy-based high-throughput assays in cystic fibrosis research

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Fluorescence microscopy is a powerful technique for advanced diagnostics procedures and addressing molecular mechanisms involved in disease, such as cystic fibrosis. Fluorescent markers targeting particular molecular species can be quantified in individual cells or cellular compartments to test disease-related cell functions. Identification of potential therapeutic targets requires batch testing of multiple treatment conditions. To increase the throughput of microscopy experiments, both image acquisition and analysis procedures need to be performed with minimal user interference.

We develop and maintain high-throughput microscopy (HTM) workflows in which both image acquisition and analysis are performed in automated fashion for large sample sets. We are currently making use of the HTM power to identify genes and compounds that can improve the function of the SLC26A9 chloride channel in the absence of CFTR. For that purpose we previously set up high-throughput measurements of apical membrane potential.

Furthermore, we apply HTM to measure enzyme activities using Forster Resonance Energy Transfer (FRET) with specialised molecular sensors. Simultaneous imaging in multiple spectral channels allows quantifying the ratio of donor and acceptor fluorescence. Disruption of sensor integrity by enzyme results in the change of the ratio. Importantly, microscopy-based assays are suitable to correlate cell morphology with functional measurements. Therefore, ratiometric FRET measurements can be performed for specific cell types even in heterogeneous diagnostic samples. Now we are optimising automated feedback microscopy protocol for automated identification of target cells during image acquisition.

Presented high-throughput image acquisition and analysis techniques are in constant use at the Imaging Core Lab at the DZL site in Heidelberg (TLRC). The generalised analysis pipeline can be further adopted for other fluorescence microscopy assays by incorporating specific functions for extracting cellular features.
Introduction

Hyperpolarized $^{129}$Xe MRI is a highly sensitive probe for regional lung function and previous work has shown the sensitivity of hyperpolarized $^{129}$Xe MRI for inflammatory changes after lung provocation by lipopolysaccharide (LPS) in animals. Purpose of this work was to investigate the feasibility of monitoring changes in regional lung function parameters after segmental LPS challenge using hyperpolarized $^{129}$Xe MRI in humans.

Methods

Thirteen healthy volunteers were recruited for this study. $^{129}$Xe MRI sessions were performed one week before and 24h after segmental LPS challenge. Bronchoalveolar lavage was performed in the challenged segment and inflammatory cells were assessed. The MR protocol included dissolved-phase imaging of $^{129}$Xe gas uptake and subsequent high-resolution ventilation imaging during the same breath-hold after inhalation of a gas mixture containing 1l hyperpolarized $^{129}$Xe. $^{129}$Xe signals were separated into gaseous $^{129}$Xe (GP), $^{129}$Xe in aqueous solution (tissue/plasma, TP), and in red blood cells (RBC). A ratio map of ventilation after provocation compared to baseline was derived. Regions of mildly decreased ventilation in the challenged segments were identified and used as regions of interest (ROIs) for data analysis.

Results

Ten volunteers successfully completed the study and were included for data analysis. The average RBC-TP ratio in the ROI applied to co-registered dissolved-phase imaging data was significantly lower after provocation ($p = 0.0039$), whereas the average TP-GP ratio was significantly elevated ($p = 0.0488$). No significant correlation of imaging-derived parameters with the amount of inflammatory cells was found after LPS challenge. There was a trend of an inverse correlation of RBC-TP and number of neutrophilic granulocytes.

Conclusion

Lung areas with segmental LPS challenge show a local reduction in the gas-up-take parameter RBC-TP, likely due to pulmonary edema causing expansion of the interstitial space in the lung parenchyma. Hyperpolarized $^{129}$Xe dissolved-phase imaging may have added value to lavage fluid analysis.
Abstract No. 905

**Impact of T2* relaxation time on quantification of regional ventilation derived by Fourier Decomposition MRI**

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**Introduction** Conventionally, T2* relaxation time is not considered in Fourier Decomposition (FD) [1] analysis. Since T2* relaxation time depends on lung volume and is not uniformly distributed within the lung [2], T2* effects on quantification of lung ventilation using FD need to be examined. Therefore, to determine lung volume dependent T2*, a triple-echo sequence was used for FD imaging. Quantification of regional lung ventilation using conventional and T2* corrected FD was compared using the mean value of fractional ventilation (FV) over the whole imaged lung volume and its distribution in anterior-posterior direction.

**Material and Methods** Twelve healthy volunteers underwent an MRI examination at 1.5 T. Images of a sagittal slice were acquired using a 2D triple-echo FLASH sequence. After registration, proton density (PD) weighted signal was calculated by a mono-exponential fit. FV and FVT2* (T2* corrected) were quantified according to Zapke et al. [3]. Mean slice FV and FVT2* values were calculated and assessed with correlation analysis and Wilcoxon signed-rank test. The gravitational dependency in anterior-posterior direction was evaluated using a linear model with an intercept.

**Results** The correlation of mean FV with FVT2* provided a slope of 0.917 (R2 = 0.975), however Wilcoxon signed-rank test found systematic differences between mean FV and FVT2* (p = 0.0005). While a linear increase towards posterior lung regions was observed for FV (slope = 0.0021 1/cm), no linear gradient in anterior-posterior direction was apparent for FVT2* (slope = -0.0003 1/cm).

**Conclusion** Although correlation analysis revealed high correlation between global FV and FVT2*, a regional difference in anterior-posterior direction is observed. This can be explained by blood distribution in supine position, which leads to a T2* gradient.

**References**


Abstract No. 906

Live tracking of wound closure in the mouse airway epithelium

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The epithelial barrier of the airways is essential to defend against pathogens. Restoring this barrier after lesions is crucial. Although epithelial repair of large epithelial wounds has been studied, information on closure of small wounds in the epithelium is missing.

To follow the wound closure of the mouse tracheal epithelium over time we lesioned small areas (1-12 cells) of the epithelium of excised mouse tracheae using a femtosecond laser and followed the wound closure up to 6 h by autofluorescence multiphoton microscopy. We also studied wound closure by scanning and transmission electron microscopy, fluorescence staining for F-actin and a proliferation assay.

Lesions with a size of up to 6 cells were closed by extension of the surrounding epithelial cells within 6 h. The damaged cells were extruded from the epithelium into the luminal side. Electron microscopy confirmed that the surrounding epithelial cells directly closed these small lesions. We did not detect proliferation of basal or adjacent ciliated or non-ciliated cells. Most lesions larger than 6 cells did not close fully in the observation period of 6 h but we observed that basal cells flattened to cover parts of the basement membrane. Delayed wound closure was partly attributable to damage of the basement membrane. Cells facing the lesion showed increased F-actin staining indicating an active cell movement toward the injury, but not all cells initially facing the lesion participated directly in wound closure. This indicates that closure is driven by movement of individual cells rather than a coordinated transepithelial process.

Small wounds in the pseudostratified airway epithelium close within hours by extension of the surrounding epithelial cells to restore epithelial barrier function. Closure of larger wounds involves basal cells.
Abstract No. 907

**Influence of Exposure Parameters and Iterative Reconstruction on MDCT-based Lung Densitometry – An ex vivo Phantom Study**

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**Objectives**
To evaluate the influence of exposure parameters and raw-data-based iterative reconstruction (IR) on lung densitometry on multidetector CT (MDCT) by dedicated in-house software.

**Material and Methods**
10 fresh porcine heart-lung-explants (4 simulated high, 6 simulated low attenuating chest wall) were prepared in an ex vivo chest phantom. MDCT scans were performed with 120kV and 80kV, each combined with 120, 60, 30 and 12mAs, and reconstructed with both filtered back projection (FBP) and IR, resulting in 16 datasets per lung. Mean air density (AD), noise and lung density (LD) were measured by automated ROI analysis with a total of 80 ROIs per dataset, with 120kV 120mAs serving as reference.

**Results**
Overall mean noise averaged for AD was 5.5± 0.9 to 69.5±2.4 HU with FBP, and 4.2± 1.1 to 49.9± 1.8HU with IR, IR reduced noise by 28 % (p<0.001). AD increased significantly with lower exposure settings, e.g. from -995.1 (reference) to -948.5 HU (80kV 12mAs) with high and low attenuating chest wall and FBP(p<0.05). Similarly, mean LD was increased from -917.6 (reference) to -902.5HU (80kV 12mAs) with high attenuating chest wall, and decreased from -920.5 (reference) to -926.6HU (80kV 12mAs) with low attenuating chest wall and FBP (p<0.05). Noise reduction by IR induced minor changes to AD and LD compared to corresponding reconstructions FBP, for example in LD with high attenuation chest wall -926.6 in FBP and -925.4 in IR at 80kV120mAs.

**Conclusions**
AD as a background signal for densitometry and LD are significantly influenced by radiation dose. Though IR effectively reduced noise at each exposure setting with high and low attenuating chest phantoms, AD and LD are not restored in a clinically meaningful magnitude. IR may thus be used in the setting of densitometry but does not correct for systematic errors in attenuation values in low-dose chest CT.
Abstract No. 908

Optimization of Signal-to-Noise ratio for improved Lung Ventilation Imaging using 19F MRI with Perfluoropropane

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Due to its low costs, high inertness and applicability at thermal polarization, in contrast to hyperpolarized gases, fluorinated gases are promising tracers for imaging lung ventilation using fluorine magnetic resonance imaging (19F MRI). However, the low spin density of an inhaled normoxic gas mixture (21% O2, 79% C3F8) leads to a relatively low signal-to-noise ratio (SNR) limiting the temporal and spatial resolution of this technique. Therefore, purpose of this work was to improve SNR of 19F MR lung imaging by choosing the ideal pulse sequence with appropriate sequence parameters according to the present relaxation times of the inhaled gas as well as the hardware limitations.

Due to the relaxation times of the inhaled normoxic fluorine gas mixture present in the human lung (T1≈T2≈16ms, T2*≈4ms), either a spoiled gradient echo sequence (spGRE) or a balanced steady-state free precession sequence (bSSFP) are likely to achieve maximum SNR. SNR per unit time is computed considering relaxation times T1, T2 and T2*, repetition and echo time TR and TE, bandwidth BW, flip angle α, RF pulse length pL, precession frequency ω and specific absorption rate SAR on the basis of signal equations hypothesized by Haase et al. and Mulkern et al. respectively.

For the hardware used in our experiments (Siemens Magnetom 1.5T with a dedicated 19F transmit and receive coil), optimal SNR was achieved with following sequence parameters using spGRE: TR=3.9ms, TE=1.9ms, α=38° and using bSSFP: TR=3.2ms, TE=1.6ms, α=34°. In the lung of a healthy volunteer, the measured increase of SNR per unit time by 18±20% of SNR optimized bSSFP compared to SNR optimized spGRE was in good agreement with the expected value of 24.7%.

In conclusion, the proposed SNR optimization of pulmonary 19F gas MRI may improve lung ventilation imaging which is essential for diagnosis and monitoring of obstructive lung diseases.
Abstract No. 909

Automatic quantification of peripheral pulmonary vessel volume for non-invasive detection of pulmonary hypertension

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Purpose: Analysis of automated quantification of peripheral pulmonary vessel volume based on pulmonary CT angiography (CTPA) for non-invasive detection of pulmonary hypertension (PH).

Methods: 93 patients underwent right heart catheterisation and CTPA for suspected PH. Patients diagnosed with CTEPH were secondarily excluded resulting in the final study population of 74 patients (66±12 years, 50 female). Pulmonary vessel volumes were automatically quantified within 10, 15 and 20 mm of the lung periphery using in-house developed software.

Results: 42 of 74 patients were diagnosed with PH (mean pulmonary arterial pressure (mPAP) 37±11 mmHg), 32 patients had normal mPAP (17±4 mmHg). The peripheral pulmonary vessel volumes were significantly enlarged in patients with PH compared to patients without PH: 31±17 vs. 19±14 cm³ within 10 mm, 59±24 vs. 42±19 cm³ within 15 mm and 86±29 vs. 66±23 cm³ within 20 mm of the lung borders (all p<0.003). ROC analysis demonstrated an AUC for the detection of PH of 0.75 (10 mm), 0.74 (15 mm) and 0.73 (20 mm). A cut-off value of 19.4 cm³ for the 10 mm peripheral vascular volume identified PH with 74% sensitivity, 72% specificity, 78% positive and 67% negative predictive value.

Conclusion: Automated quantification of peripheral pulmonary vessel volume based on CTPA is feasible and showed significantly enlarged vessel volumes in patients with confirmed PH. Highest diagnostic accuracy was reached by the pulmonary vessel volume within 10 mm of the lung periphery. The technique may facilitate non-invasive detection of PH.
Abstract No. 910

The human alveolar epithelial type I cell reconstructed in 3D – more than a simple squamous cell

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The alveolar surface of the lung is covered by alveolar epithelial type 1 (AE1) and alveolar epithelial type 2 cells. AE1 cells cover ~97% of this surface by thin cytoplasmic extensions to enable efficient gas exchange. Former studies, primarily based on transmission electron microscopy, indicated that AE1 cells may be branched cells capable of crossing the interalveolar septa and may even have more than one apical surface. The aim of the present study was to explore their spatial complexity via serial block-face scanning electron microscopy (SBF-SEM) and three-dimensional (3D) reconstructions in archival material from a human lung (Gehr et al. Respir Physiol. 32:121-140, 1978) which was fixed via intratracheal installation 30min after death by buffered glutaraldehyde and removed afterwards during autopsy. A sample kept in fixative was specifically prepared for SBF-SEM according to Deerinck et al. (Microsc Microanal. 16:1138-1139, 2010), a protocol including ferrocyanide-reduced osmium tetroxide, thiocarbohydrazide-osmium tetroxide (OTO), uranyl acetate and lead aspartate and scanned with a Zeiss Merlin VP Compact with Gatan 3View. An image stack comprising 2046 images with a side length of (6144x6144) pixel and a pixel size of 18.5nm was generated (section thickness: 80nm). From this stack a substack of 901 images comprising a volume of 110x105x72µm³ was prepared for segmentation and modelling in IMOD (Kremer et al. J Struct Biol. 116,71-76, 1996). We were able to reconstruct three entire AE1 cells, confirm previous concepts, put them in a 3D context and discover special features such as AE1 “self-junctions”, an entire nucleus “migrating” through the alveolar wall and local aggregations of mitochondria suggesting the support of energy supply in the periphery while the overall blood gas barrier can be kept thin. These 3D reconstructions provide new insights into the complexity of the alveolar epithelium to better understand development and repair and optimize therapeutic strategies.
Abstract No. 911

Towards 3D in-vivo imaging of bronchial mucus transport by a novel all fiber based endoscope

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Several lung diseases such as cystic fibrosis, COPD or primary ciliary dyskinesia are associated with the inhibition of mucociliary clearance of the lungs. Increasingly successful research into personalized medicine calls for improved therapy control. Currently, only general markers such as improvement of lung function are clinically available. To overcome this and get direct mechanistic feedback on the impact of new therapies, a flexible endoscopic imaging procedure is needed to directly visualize ciliary function and mucus transport by high-speed and high-resolution imaging. With resolution of 10 µm and video-rate imaging of volumes, optical coherence tomography (OCT) is ideally suited for this task.

Here we present a piezo-based fiber scanning endoscope with only 1.6 mm outer diameter and 10 mm rigid length that fits through the working channel of a flexible bronchoscope. The endoscope uses a focusing optics integrated into the 2-D moving fiber to reduce the length and parasitic reflections between the scanning fiber and the imaging optics. Using an FDML-based OCT with 3.25 MHz A-scan rate, we achieved a densely sampled volumetric imaging with up to 15 volumes per second. The novel optics achieved an isotropic resolution of 10 µm at 2.8 mm³ field of view.

The combination of the miniaturized fast scanning flexible endoscope with MHz OCT shall enable comprehensive measurement of the mucus transport in the lower airways in healthy volunteers and patients.
Abstract No. 912

High-resolution spatial mapping of cellular locations and interactions in a 3D ex-vivo early fibrosis model

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Recent advances in single-cell RNA-sequencing (scRNAseq) pave the way to study and identify cell types on the basis of their gene expression profiles out of the heterogeneity found in multicellular organisms and organs. Upon sample preparation for scRNAseq the tissue is dissociated and the spatial information about the cells in-situ location is lost. Therefore, in order to validate scRNAseq data and relink the gene expression data to the spatial location of specific cell types in the lung parenchyma, we applied high-resolution confocal image acquisition of 300 µm thick precision cut lung slices (PCLS). Of note, ex-vivo cultured PCLS derived from mouse lungs provide an arising and exciting new 3D cell culture technology, which closely mimics the complexity, biomechanics and composition of in-situ lungs. With a special focus on identifying novel mesenchymal cell populations, which are involved in the onset and progression of lung fibrosis, we used specific markers retrieved out of the scRNAseq data, for the identification and location of these cell populations in the large 3D volume of PCLS. The complete 3D volume comprises multiple layers of entire alveoli, bronchioles and interstitial space. Furthermore, by treating the PCLS with TGFβ1 for 72 hours, we successfully triggered the transdifferentiation of fibroblasts into alpha-smooth muscle actin expressing myofibroblasts. Additionally, within the TGFβ1 treated PCLS we found the deposition of fibrosis- relevant insoluble extracellular matrix (ECM) proteins such as Emilin-2 and Collagen-1, as well as ECM associated proteins like SFRP1. Thus, we conclude that we can use this model, by means of PCLS derived from various fluorescent reporter mice together with live immunostainings, high-resolution 4D fluorescence confocal and lightsheet microscopy, to study the dynamics and interactions of lung cells in early fibrotic events in real time.
Abstract No. 913

3D T1 mapping in the lungs during free breathing using asymmetrical cylindrical encoding

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In MRI, the T1 relaxation time was found to be a diagnostically interesting parameter in COPD and CF patients, especially for oxygen enhanced imaging. Look-Locker inversion recovery (IR) sequence based methods for T1 mapping during free breathing and using ultrashort TE have been introduced, but have so far been limited to single slices. As acquiring multiple individual slices is time-consuming, the purpose of this work was to implement a 3D equivalent.

All measurements were performed on a 1.5T clinical scanner. The measurement consists of asymmetrical radial projections acquired after an inversion pulse, arranged using a modified golden angle increment. For 3D imaging, cylindrical encoding with 28 cartesian phase encoding steps was used. A healthy volunteer was examined, employing first a conventional linear ordering for 3D phase encoding steps, and then an ordering designed specifically for this application. Gating was applied using the k-space centre MR (DC) signal, employing a compensation for IR, as previously described. From these radial projections, a 4D set of images was reconstructed and 3D set of T1 maps were fitted per voxel. Total measurement time was 11min 15s.

The reconstructed T1 maps show successful gating but also inhomogenous T1 in the volunteer maps when using linear ordering. The optimized ordering shows homogeneous T1 maps, as expected.

This implementation demonstrates that T1 maps of the whole lung can be acquired in reasonable time during free breathing at a partition resolution comparable to 2D single slice measurements. 3D encoding reduces time resolution of the gating signal and expands the volume the DC-signal is received from, but the IR correction remains applicable.

However, gating is vulnerable to the order in which k-space is acquired: Acquiring phase encoding steps in linear ordering during IR leads to artefacts in the resulting T1 maps, but using the optimized scheme mitigates this.
Abstract No. 914

Feasibility of Quantitative Regional Ventilation and Perfusion Mapping with Phase-Resolved Functional Lung (PREFUL) MRI in Healthy Volunteers and COPD, CTEPH, and CF Patients

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Introduction: Fourier Decomposition (FD) is a MRI method for simultaneous lung perfusion (Q) and ventilation (V) assessment¹. Due to its free-breathing acquisition, no requirement for additional hardware and radiation-free V/Q measurement, FD is a promising candidate for clinical translation. Although many phases are acquired, the conventional analysis considers only specific ventilation- and perfusion phases. Using ultra-fast sequences² or self-gating³ regional increase of perfusion-related phase was shown in patients with cystic fibrosis (CF)²,⁴. In this study, a phase-resolved functional lung imaging (PREFUL⁵) post-processing method is introduced and tested in patients with pulmonary disease.

Methods: Gradient-echo MR image time series with a temporal resolution of ~300 ms of two healthy volunteers, one chronic thromboembolic hypertension (CTEPH), one CF, and one chronic obstructive pulmonary disease (COPD) patient were acquired at 1.5 T. A model function was used to estimate the cardiac and respiratory phase. After interpolation to an equidistant time grid, full cardiac and respiratory cycles were obtained. Ventilation and perfusion related time to peak (V-TTP/Q-TTP), fractional ventilation (FV) and Q-weighted maps, respective V/Q, and FV flow-volume loops were calculated.

Result: Homogenous V-TTP and Q-TTP maps were obtained for the volunteers. Q-TTP was increased in hypo-perfused regions of the CTEPH patient in agreement with dynamic contrast enhanced MRI and improved after postpulmonary endarterectomy surgery. Patterns of increased Q-TTP and V-TTP in hypo-perfused and hypo-ventilated regions were found for the CF and COPD patient. The FV flow-volume loops of the COPD patient were smaller in comparison with the healthy volunteers, and showed regional differences in agreement with CT.

Conclusion: It is feasible to obtain complementary dynamic perfusion and ventilation information with conventional FD acquisition using PREFUL.

References: ¹Bauman et al. (2009), MRM-62(3):656-664; ²Bauman et al. (2012), ISMRM-1340; ³Fischer et al. (2014), NMR in Biomedicine-27(8):907-917; ⁴Veldhoen et al. (2016), ISMRM-1144; ⁵Voskrebenzev et al. (2017), MRM.
Abstract No. 915

High-Resolution X-Ray Microtomography for the Detection of Low-Grade Pulmonary Inflammation in MGL-tg Mice

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Nuclear suppressor of cytokine signaling 1 (SOCS1) is a proposed regulator of local lung immunity. SOCS1, a negative feedback inhibitor of cytoplasmic Janus kinase, signal transducer and activator of transcription signaling contains a nuclear localization sequence (NLS). Mice fully deficient for SOCS1 die within 3 weeks after birth due to multiorgan inflammation, mice expressing only non-nuclear Socs1ΔNLS (Socs1−/−MGL-tg mice) are rescued from early lethality and develop low-grade lung inflammation.

To characterize morphological lung abnormalities in MGL-tg mice, 8 weeks old animals were examined by high-resolution micro computed tomography (µ-CT) (spatial resolution:18µm) directly after euthanization. Two independent readers identified three distinct abnormal morphological features in MGL-tg lungs compared with wild type (wt) mice. First, focal circular nodularities, predominantly perivascular in distribution. Second, diffuse ground-glass-like opacifications. Third, peribronchial cuffing (subtle peribronchial wall thickening) the latter not reaching statistically significant difference between MGL-tg and wt mice. Subsequently single focal circular nodularities were segmented to obtain volumetric data on the inflammatory burden.

After direct post mortem µ-CT assessment, lungs were fixed by tracheal instillation of formalin and embedded in paraffin. Subsequent µ-CT scans acquired with an optimized protocol (spatial resolution: 9µm) of paraffin embedded lungs were obtained to generate maps for µ-CT-guided tissue sectioning, to facilitate direct micro-radiologic to histopathologic correlation. The focal circular nodularities correlated directly to round perivascular aggregates of lymphocytes. Diffuse ground-glass like opacifications correlated to amorphous eosinophilic hyaline material in alveolar spaces without the presence of inflammatory cells, suggesting a possible morphological correlate to a suggested disrupted epithelial cell barrier, described in MGL-tg mice.

Our study demonstrates that µ-CT is a sensitive technique for early detection of morphological abnormalities of low-grade inflammation in the murine lung. Furthermore, µ-CT-examination of paraffin embedded lung tissue may be used in the mapping for subsequent direct micro-radiologic to histopathologic correlation.
COPD phenotyping with parameter response maps based on paired inspiratory/expiratory low-dose computed tomography

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Objectives: Patients suffering from chronic obstructive pulmonary disease (COPD) can be subdivided using computed tomography (CT) into an airway-dominant or emphysema-dominant phenotype. The parametric response map (PRM) method, a voxel-wise image analysis technique may detect distinct functional differences between these phenotypes.

Methods and Materials: 528 paired inspiratory/expiratory low-dose CT scans (120 kV, 30-35 mAs, ~3.5 mSv) from the multicenter COSYCONET study were visually classified as airway-dominant (60%) and emphysema-dominant (40%). PRM was computed fully automatically employing a deformable volumetric registration and lung segmentation, classifying lung voxels as normal parenchyma (PRMNormal), functional small-airways disease (PRMfSAD) and emphysema (PRMEmph). For comparison the emphysema index (EI) was calculated on the inspiratory scans.

Results: Average PRMNormal, PRMfSAD, PRMEmph and EI were 66.5%, 25.9%, 6.7% and 9.3% in the airway-dominant group and 44.1%, 33.2%, 21.6% and 25.3% in the emphysema-dominant group, respectively. The differences were significant between the groups (p < 1.1e-10 for all). The Pearson correlation coefficient r between PRMNormal and forced expiratory volume in 1s (FEV₁) was 0.62, for PRMfSAD -0.52, for PRMEmph -0.55 and for EI -0.55 (p < 2.2e-16 for all) across all patients, which was similar for airway-dominant and emphysema-dominant phenotypes.

Conclusion: PRM identified fSAD regions, which would have been missed by emphysema quantification just on inspiratory CT scans. The results show that in emphysema-dominant COPD there is a large fraction of fSAD besides irreversible emphysema, which may be addressed by therapy. Additionally, the combination of PRMfSAD and PRMEmph leads to a higher correlation with FEV₁.
Volume Electron Microscopy of Human Lung Tissue

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Volume electron microscopy allows the generation of 3D volume datasets of different size and resolution, down to nanometre scale. Samples can be visualized without the need for specific staining of selected targets. Thus, an essential prerequisite is given to understand cellular processes: The naive observation of the full structural context on an ultra-structural level. Datasets produced by Serial-Block-Face-Scanning-Electron-Microscopy (SBF-SEM) can be read back and forth over and over again like exciting novels, giving rise to details concerning the connectivity and composition of cellular frameworks. We show here datasets of healthy and fibrotic human lung tissue.
Author Index
<table>
<thead>
<tr>
<th>First Name</th>
<th>Last Name</th>
<th>Abstract No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Till</td>
<td>Acker</td>
<td>H</td>
</tr>
<tr>
<td>Mania</td>
<td>Ackermann</td>
<td>205</td>
</tr>
<tr>
<td>Lorenz</td>
<td>Adlung</td>
<td>701</td>
</tr>
<tr>
<td>Alisa</td>
<td>Agné</td>
<td>101*</td>
</tr>
<tr>
<td>Agustin</td>
<td>Agustin Rodriguez-Gonzalez</td>
<td>701*</td>
</tr>
<tr>
<td>Marjan</td>
<td>Ahmadi</td>
<td>001*</td>
</tr>
<tr>
<td>Negah</td>
<td>Ahmadvand</td>
<td>601*</td>
</tr>
<tr>
<td>Peter</td>
<td>Ahnert</td>
<td>308,314</td>
</tr>
<tr>
<td>Nancy</td>
<td>Ahrendt</td>
<td>441</td>
</tr>
<tr>
<td>Linda</td>
<td>Ahrens</td>
<td>606</td>
</tr>
<tr>
<td>Saket</td>
<td>Ahuja</td>
<td>417</td>
</tr>
<tr>
<td>Michaela</td>
<td>Aichler</td>
<td>406</td>
</tr>
<tr>
<td>Andres</td>
<td>Alberro-Brage</td>
<td>301*</td>
</tr>
<tr>
<td>Melanie</td>
<td>Albrecht</td>
<td>602*</td>
</tr>
<tr>
<td>Tobias</td>
<td>Albrecht</td>
<td>221</td>
</tr>
<tr>
<td>Nasim</td>
<td>Alebrahimdehkordi</td>
<td>F,501*</td>
</tr>
<tr>
<td>Francesca</td>
<td>Alessandrini</td>
<td>026</td>
</tr>
<tr>
<td>Goesmann</td>
<td>Alexander</td>
<td>801</td>
</tr>
<tr>
<td>Nadine</td>
<td>Alfeis</td>
<td>201*,215,224</td>
</tr>
<tr>
<td>Fahd</td>
<td>Alhamdan</td>
<td>021</td>
</tr>
<tr>
<td>Kristina</td>
<td>Alikhanyan</td>
<td>702*</td>
</tr>
<tr>
<td>Hani</td>
<td>Alsafadi</td>
<td>408</td>
</tr>
<tr>
<td>Peter</td>
<td>Alter</td>
<td>110,111</td>
</tr>
<tr>
<td>Tufman</td>
<td>Amanda</td>
<td>734</td>
</tr>
<tr>
<td>Ole</td>
<td>Ammerpohl</td>
<td>735</td>
</tr>
<tr>
<td>Vasileios</td>
<td>Andrianopoulos</td>
<td>120</td>
</tr>
<tr>
<td>Iliaas</td>
<td>Angelidis</td>
<td>448,912</td>
</tr>
<tr>
<td>Siddhesh</td>
<td>Aras</td>
<td>F</td>
</tr>
<tr>
<td>Kristina A. M.</td>
<td>Arendt</td>
<td>703,720,724,726</td>
</tr>
<tr>
<td>Vasileios</td>
<td>Armenis</td>
<td>703,720</td>
</tr>
<tr>
<td>Stefan</td>
<td>Arnhold</td>
<td>106</td>
</tr>
<tr>
<td>Paola</td>
<td>Arnold</td>
<td>502,608</td>
</tr>
<tr>
<td>Ingolf</td>
<td>Askevold</td>
<td>322</td>
</tr>
<tr>
<td>Yassen</td>
<td>Assenov</td>
<td>729</td>
</tr>
<tr>
<td>Katrin Julia</td>
<td>Audrit</td>
<td>002*</td>
</tr>
<tr>
<td>Murat</td>
<td>Avsar</td>
<td>606</td>
</tr>
<tr>
<td>Jens</td>
<td>Axmann</td>
<td>521</td>
</tr>
<tr>
<td>Anna</td>
<td>Bach</td>
<td>423</td>
</tr>
<tr>
<td>Thomas</td>
<td>Bahmer</td>
<td>A,003*,007,012,014,016,022,029,0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>36,037,038,449</td>
</tr>
<tr>
<td>Anita</td>
<td>Balazs</td>
<td>903</td>
</tr>
<tr>
<td>Robert</td>
<td>Bals</td>
<td>110,111,801,803</td>
</tr>
<tr>
<td>Anita</td>
<td>Balázs</td>
<td>202*</td>
</tr>
<tr>
<td>Michaela</td>
<td>Barnikel</td>
<td>429,502*,608</td>
</tr>
<tr>
<td>Guillermo</td>
<td>Barreto</td>
<td>405,438,440,456,719, 730,735</td>
</tr>
<tr>
<td>Margarida</td>
<td>Barroso</td>
<td>302*</td>
</tr>
<tr>
<td>Sabine</td>
<td>Bartel</td>
<td>004*,013,015,023,114,121</td>
</tr>
<tr>
<td>Grit</td>
<td>Barton</td>
<td>303*</td>
</tr>
<tr>
<td>Sandra</td>
<td>Barth</td>
<td>C,J</td>
</tr>
<tr>
<td>First Name</td>
<td>Last Name</td>
<td>Abstract No.</td>
</tr>
<tr>
<td>-----------</td>
<td>----------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Marek</td>
<td>Bartkuhn</td>
<td>801</td>
</tr>
<tr>
<td>Jürgen</td>
<td>Barton</td>
<td>502,608</td>
</tr>
<tr>
<td>Yannic</td>
<td>Bartsch</td>
<td>025</td>
</tr>
<tr>
<td>Carl-Peter</td>
<td>Bauer</td>
<td>010</td>
</tr>
<tr>
<td>Michael</td>
<td>Bauer</td>
<td>308,314</td>
</tr>
<tr>
<td>Uta-Maria</td>
<td>Bauer</td>
<td>504</td>
</tr>
<tr>
<td>Franz</td>
<td>Bauernfeind</td>
<td>E</td>
</tr>
<tr>
<td>Ingo</td>
<td>Baumann</td>
<td>221</td>
</tr>
<tr>
<td>Ulrich</td>
<td>Baumann</td>
<td>218</td>
</tr>
<tr>
<td>Sandra</td>
<td>Baus</td>
<td>603*</td>
</tr>
<tr>
<td>Lukas</td>
<td>Becker</td>
<td>531*</td>
</tr>
<tr>
<td>Stephan</td>
<td>Becker</td>
<td>323</td>
</tr>
<tr>
<td>Johannes</td>
<td>Beckers</td>
<td>108</td>
</tr>
<tr>
<td>Frauke</td>
<td>Beckert</td>
<td>101</td>
</tr>
<tr>
<td>Mariola</td>
<td>Bednorz</td>
<td>106,109</td>
</tr>
<tr>
<td>Frederik</td>
<td>Behr</td>
<td>218</td>
</tr>
<tr>
<td>Jürgen</td>
<td>Behr</td>
<td>110,111,119,408,409,410,421,422,425,428,429,437,502,608</td>
</tr>
<tr>
<td>Jochen</td>
<td>Behrends</td>
<td>004</td>
</tr>
<tr>
<td>Lea</td>
<td>Behrendt</td>
<td>901*,905</td>
</tr>
<tr>
<td>Petra</td>
<td>Behrens</td>
<td>011</td>
</tr>
<tr>
<td>Lukas</td>
<td>Beike</td>
<td>420</td>
</tr>
<tr>
<td>Saverio</td>
<td>Bellusci</td>
<td>109,307,328,405,418,432,454,601</td>
</tr>
<tr>
<td>Nicola</td>
<td>Benjamin</td>
<td>107,506,507,511</td>
</tr>
<tr>
<td>Dietrich</td>
<td>Berdel</td>
<td>010</td>
</tr>
<tr>
<td>Wilhelm</td>
<td>Bertrams</td>
<td>102*,312,324,331</td>
</tr>
<tr>
<td>Anita</td>
<td>Bhandari</td>
<td>118</td>
</tr>
<tr>
<td>Frank</td>
<td>Bieritz</td>
<td>110,111</td>
</tr>
<tr>
<td>Heike</td>
<td>Biller</td>
<td>904</td>
</tr>
<tr>
<td>Leonhard</td>
<td>Binzenhöfer</td>
<td>437</td>
</tr>
<tr>
<td>Surinder</td>
<td>Birring</td>
<td>449</td>
</tr>
<tr>
<td>Elena</td>
<td>Bischoff</td>
<td>107</td>
</tr>
<tr>
<td>Rainer</td>
<td>Blasczyk</td>
<td>605</td>
</tr>
<tr>
<td>Rebecca</td>
<td>Bodenstein-Sgro</td>
<td>005*, 024</td>
</tr>
<tr>
<td>Elinor</td>
<td>Boos</td>
<td>710</td>
</tr>
<tr>
<td>Paul</td>
<td>Borchert</td>
<td>503*</td>
</tr>
<tr>
<td>Tilman</td>
<td>Borggrefe</td>
<td>H</td>
</tr>
<tr>
<td>Tina</td>
<td>Bormann</td>
<td>401*</td>
</tr>
<tr>
<td>Judith</td>
<td>Bossen</td>
<td>032,704*</td>
</tr>
<tr>
<td>Sebastien</td>
<td>Boutin</td>
<td>203*,204*,801</td>
</tr>
<tr>
<td>Anrdzej</td>
<td>Boznanski</td>
<td>019</td>
</tr>
<tr>
<td>Farastuk</td>
<td>Bozorgmehr</td>
<td>709</td>
</tr>
<tr>
<td>Dionne</td>
<td>Braeken</td>
<td>305*</td>
</tr>
<tr>
<td>Christina</td>
<td>Brandenberger</td>
<td>306*</td>
</tr>
<tr>
<td>Ralf</td>
<td>Brandes</td>
<td>F</td>
</tr>
<tr>
<td>Peter</td>
<td>Braubach</td>
<td>033,116,413,602,612, 717</td>
</tr>
<tr>
<td>Armin</td>
<td>Braun</td>
<td>006,033,116,330,413,717</td>
</tr>
<tr>
<td>Thomas</td>
<td>Braun</td>
<td>302,405,438,454,501,730</td>
</tr>
<tr>
<td>Charlotte</td>
<td>Braun-Fahrländer</td>
<td>019</td>
</tr>
<tr>
<td>Corinna</td>
<td>Brehm</td>
<td>H,705</td>
</tr>
<tr>
<td>Anna</td>
<td>Brichkina</td>
<td>705*</td>
</tr>
<tr>
<td>Kerstin</td>
<td>Brinkert</td>
<td>205*</td>
</tr>
<tr>
<td>David</td>
<td>Brunn</td>
<td>706*</td>
</tr>
<tr>
<td>First Name</td>
<td>Last Name</td>
<td>Abstract No.</td>
</tr>
<tr>
<td>------------</td>
<td>---------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Theresa</td>
<td>Buchegger</td>
<td>205</td>
</tr>
<tr>
<td>Michael</td>
<td>Buchholz</td>
<td>J</td>
</tr>
<tr>
<td>Scott</td>
<td>Budinger</td>
<td>D</td>
</tr>
<tr>
<td>Carmen</td>
<td>Buettner</td>
<td>113</td>
</tr>
<tr>
<td>Bernd</td>
<td>Bufe</td>
<td>319</td>
</tr>
<tr>
<td>Martin</td>
<td>Buhmann</td>
<td>521</td>
</tr>
<tr>
<td>Dmitri</td>
<td>Bulavin</td>
<td>705</td>
</tr>
<tr>
<td>Janette</td>
<td>Burgess</td>
<td>A1,A2</td>
</tr>
<tr>
<td>Gerald</td>
<td>Burgstaller</td>
<td>408,445,912</td>
</tr>
<tr>
<td>Andrew</td>
<td>Bush</td>
<td>455</td>
</tr>
<tr>
<td>Alena</td>
<td>Buyx</td>
<td>I</td>
</tr>
<tr>
<td>Danalyn</td>
<td>Byng</td>
<td>103*</td>
</tr>
<tr>
<td>Hans Peter</td>
<td>Bächinger</td>
<td>437</td>
</tr>
<tr>
<td>Diana</td>
<td>Böhm</td>
<td>304*</td>
</tr>
<tr>
<td>Mario</td>
<td>Böhm</td>
<td>523</td>
</tr>
<tr>
<td>Martin</td>
<td>Böhm</td>
<td>701,732</td>
</tr>
<tr>
<td>Andreas</td>
<td>Böning</td>
<td>101</td>
</tr>
<tr>
<td>Marisa</td>
<td>Böttger</td>
<td>020</td>
</tr>
<tr>
<td>Jannie Marie</td>
<td>Bülow Sand</td>
<td>A</td>
</tr>
<tr>
<td>Hector</td>
<td>Cabrera-Fuentes</td>
<td>438</td>
</tr>
<tr>
<td>Brian</td>
<td>Caffrey</td>
<td>312</td>
</tr>
<tr>
<td>Julia</td>
<td>Camargo Neumann</td>
<td>602</td>
</tr>
<tr>
<td>A.</td>
<td>Cardenas-Blanco</td>
<td>612</td>
</tr>
<tr>
<td>Alfonso</td>
<td>Carleo</td>
<td>402*,403*</td>
</tr>
<tr>
<td>Gianni</td>
<td>Carraro</td>
<td>307</td>
</tr>
<tr>
<td>Saskia</td>
<td>Carstensen</td>
<td>104*</td>
</tr>
<tr>
<td>Marco</td>
<td>Carvalho Oliveira</td>
<td>605</td>
</tr>
<tr>
<td>Noemi</td>
<td>Casteletti</td>
<td>707*</td>
</tr>
<tr>
<td>Felix</td>
<td>Ceelen</td>
<td>429,502,608</td>
</tr>
<tr>
<td>Adam</td>
<td>Chaker</td>
<td>003,026,028,037,038</td>
</tr>
<tr>
<td>Trinad</td>
<td>Chakraborty</td>
<td>308</td>
</tr>
<tr>
<td>Cho-Ming</td>
<td>Chao</td>
<td>307*, 328, 405, 432, 601</td>
</tr>
<tr>
<td>Prakash</td>
<td>Chelladurai</td>
<td>440,504*,508,738</td>
</tr>
<tr>
<td>Rongjun</td>
<td>Chen</td>
<td>329</td>
</tr>
<tr>
<td>Yuanyuan</td>
<td>Chen</td>
<td>708*</td>
</tr>
<tr>
<td>Chen</td>
<td>Chen-Wacker</td>
<td>605</td>
</tr>
<tr>
<td>Shashi</td>
<td>Chillappagari</td>
<td>411,417,423</td>
</tr>
<tr>
<td>Philippe</td>
<td>Chouvarine</td>
<td>212</td>
</tr>
<tr>
<td>Sandra</td>
<td>Christochowitz</td>
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