ABSTRACT BOOK

11th DZL Annual Meeting | 14–16 June 2023

MUNICH SITE

Deutsches Zentrum für Lungenforschung e. V
Geschäftsstelle | Aulweg 130 | 35392 Gießen

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You can find the full program and all further information about the DZL Annual Meeting 2023 on our event page.

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Teaser Sessions Schedule

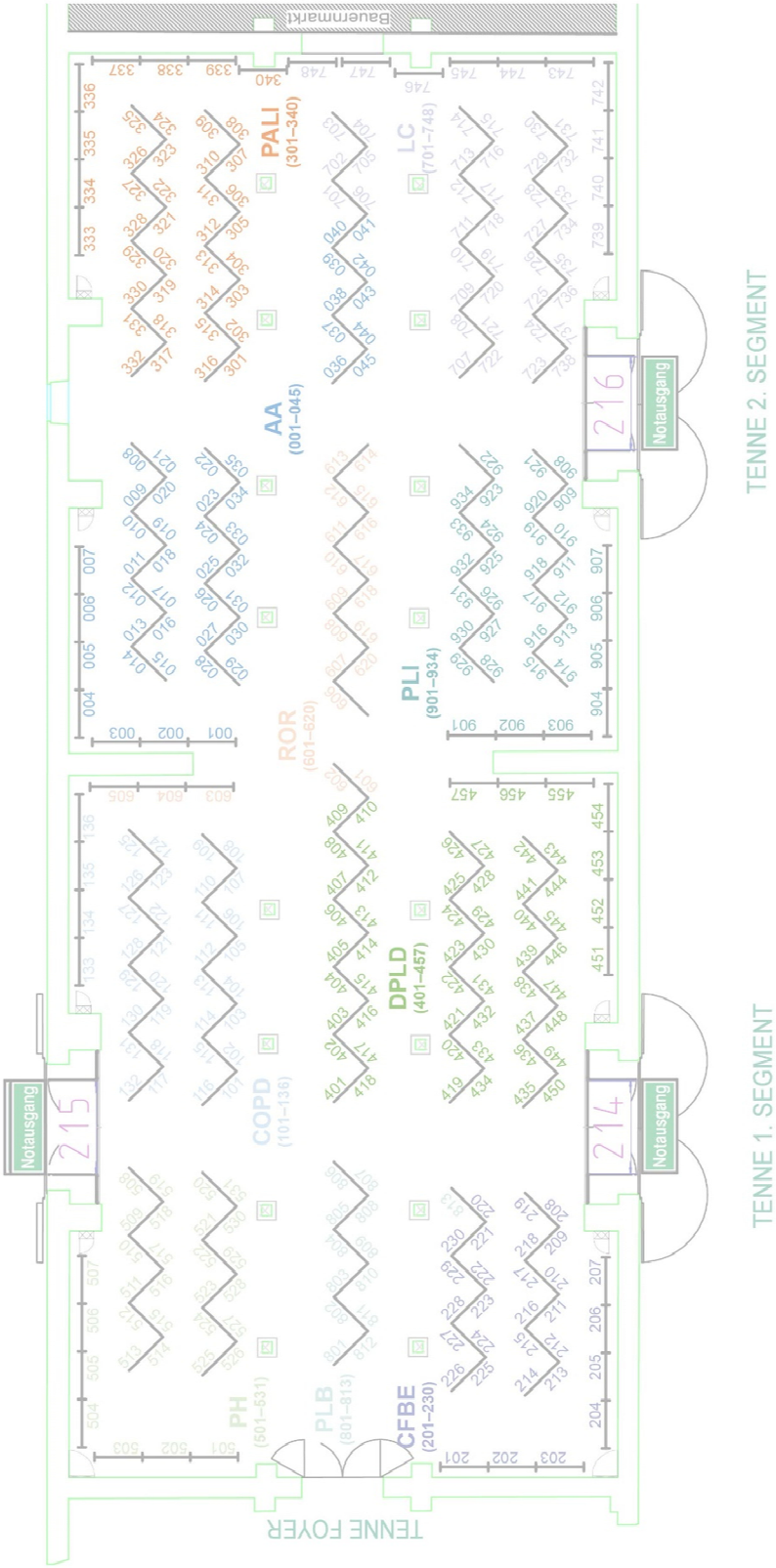
June 15th, 2 p.m. – 3 p.m.
Presentations: 2 mins / change time 1 min

	Stadtsaal	Säulensaal	Kleiner Saal	SR 1	SR 5	
14:00	001	404	302	203	902	14:00
14:03	003	406	309	206	907	14:03
14:06	006	414	310	208	910	14:06
14:09	013	415	312	210	912	14:09
14:12	014	418	314	216	916	14:12
14:15	015	420	317	217	919	14:15
14:18	021	422	321	218	920	14:18
14:21	022	423	323	223	922	14:21
14:24	026	436	331	224	928	14:24
14:27	041	447	337	225	932	14:27
14:30	106	503	701	601	802	14:30
14:33	108	507	702	603	803	14:33
14:36	110	509	705	604	804	14:36
14:39	112	514	714	606	805	14:39
14:42	114	515	722	608	806	14:42
14:45	119	516	723	609	809	14:45
14:48	120	518	724	611	810	14:48
14:51	123	520	728	619	811	14:51
14:54	129	524	736	620		14:54
14:57	130	528	738			14:57

Poster-No.	Disease Area	Adminstrative Coordinator:
000	AA	Dr. Jörn Bullwinkel
100	COPD	Dr. Jörn Bullwinkel
200	CFBE	Dr. Annegret Zurawski
300	PALI	Dr. Sylvia Weißmann
400	DPLD	Franziska Hauptkorn, Dr. Jutta Schlegel
500	PH	Dr. Sylvia Weißmann
600	ROR	Dr. Annegret Zurawski
700	LC	Dr. Birgit Teucher
800	PLB	Dr. Jutta Schlegel
900	PLI	Dr. Birgit Teucher

Poster Session Floorplan

June 15th, 3 p.m. – 5 p.m., Tenne EG



DA Asthma and Allergy: Abstract No. 001 – 045

Abstract No. 001

Trainable ECM genes in asthma development

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Background:
Rhinoviruses (HRV) are considered the most frequent viruses involved in human's common cold illness. They trigger the upper respiratory illnesses in non-asthmatic and the lower respiratory illnesses in asthmatic children and adults. The regulation of extracellular matrix (ECM) proteins during asthma development is still not fully elucidated. However, identifying the trainable genes related to ECM remodeling will contribute to a better understanding of asthma progression and possibly helps to prevent.
Methods:
The experiment consisted of repeating HRV-16 infections of Human bronchial epithelial cells (BEAS-2B). After the first infection cells were split into two parts (RNA and DNA), each part was reseeded up to five infections. The genome-wide DNA methylation and mRNA expression were analyzed for the first, third and fifth infection samples. A mixed model was used to integrate the results and predict the trained genes.
Results:
Through the analysis we identified 66 DEGs that share roles in basement membrane organization, this include the keratan sulfate metabolism, heparan sulfate metabolism, chondroitin sulfate metabolism, ECM proteoglycans, collagen formation, laminin interactions and elastic fiber formation. We predicted 24 genes related to basement membrane to be trained. Our results showed the regulatory roles of DNA methylation on trained basal cell membrane genes such as (*HS6ST1*;FC =-1.20, *B3GAT2*; FC =-1.30, *COL1A2*; FC =-2.66, *LAMB3*; FC =2.50, *LAMC2*; FC =2.07, *MFAP4*; FC =-2.02, *FBN1*; FC =-1.51).
Conclusions:
The inflammatory responses in asthmatic patients are correlated with basement membrane protein dysregulation. We found 24 trained genes in the basement membrane. The future treatment could be linked with targeting the proteins encoded by those genes.

Abstract No. 002

Immunomodulatory and anti-viral effects of CDE in RSV-infected human lung tissue ex vivo

Mareike Ahrends[1], **Valerie Beneke**[1], Franka Hage[1], Olga Danov[1], Laurin Büning[1], Bettina Rankl[2], Christopher Werlein[3], Patrick Zardo[3], Armin Braun[1], Christoph Müller[4], Danny Jonigk[5], Erika von Mutius[2], Katherina Sewald[1]

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The Respiratory Syncytial Virus (RSV) is a common cause of acute respiratory tract infections and is linked to the onset and exacerbation of chronic lung diseases, such as asthma. Currently, there is no causal treatment for RSV infections and no approved vaccine for active immunization. To investigate the immunological reaction to RSV, human-based models are preferred for non-clinical studies. Here, we tested the effect of cow shed dust extract (CDE) on RSV infections and the local immune response in human precision-cut lung slices (PCLS).

Human PCLS were either pre-treated with CDE for 1 hour and subsequently infected with RSV for 2 hours or post-treated with CDE after infection. CDE treatment was continued for up to 3 days. Tissue viability was measured by LDH- and WST-1 assay. Viral load was determined by qRT-PCR. Immunological changes induced by viral infection and CDE treatment were analyzed by multiplex ELISA.

CDE alone did not show any toxic effects in human lung tissue and upregulated secretion of multiple pro-inflammatory cytokines, including IFN-γ (10-fold), TNF-α (18-fold), IL-17A (5-fold) and IL-8 (2-fold). Further, it reduced RSV load (67 % ± 21 % reduction at 100 µg/mL) and subsequently virus-associated cytokines in PCLS (IFN-γ: 4-fold, IP-10: 4-fold, IFN-α: 42-fold reduction). Therapeutic treatment was more effective than pre-treatment, whereas a combination of both was most effective.

Overall, CDE represents a promising novel therapeutic option for RSV infections. To gain mechanistical understanding, we are currently investigating TLR antagonists in combination with CDE. Further, the effect of CDE on allergic airway responses, which have been described to be linked to RSV infections, will be tested in an HDM-induced asthma model *in vivo*.

Abstract No. 003

Transcriptional gene regulation of Interleukin-6 in bronchial epithelial cells in the context of viral infections and chronic lung diseases

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Background: Viral infections can lead to acute exacerbations in chronic lung diseases (CLD) such as asthma. PR domain zinc finger protein 1 (PRDM1) previously was shown to repress Interleukin-6 (IL-6) gene expression. However, IL-6 gene regulation in epithelial cells during viral infections is only partially understood. **Objective**: We hypothesize that the IL-6 gene regulation is counter-regulated in epithelial cells after viral analog stimulation. **Methods**: ENCODE data sets were used to identify TFs binding in the IL-6 promoter. Chromatin immunoprecipitation sequencing (ChIP-Seq) of TFs PRDM1 (clone 9115BF) and Interferon regulatory factor 1 (IRF1; clone ab240299) was performed in diseased BEC (Lonza) after 3h polyinosinic:polycytidylic acid (PIC) stimulation. A549 stimulated with 10 µg/mL PIC were harvested in a 30-minute interval for 4h. Intersection point calculation of linear graphs determined an approximate time point of stationary phase onset. **Results**: IL-6 regression models revealed an association with two TFs (IFR1: $p<0.001$, $r2=0.85$; PRDM1: $p<0.001$, $r2=0.86$, verified by ChIP-Seq). Spearman correlation revealed a stronger binding of PRDM1 in the IL-6 gene ($p>0.0003$) compared to IRF-1 ($p>0.0465$). Expression curves of IL-6 and PRDM1 displayed similar sigmoid shapes while IRF-1 resembled a steady increase. PRDM1 gene expression ($x=1.76h$) reached the stationary phase earlier than IL-6 ($x=1.99h$). **Conclusion**: PRDM1 and IRF1 bind significantly in the 3'-UTR of the IL-6 gene. Similar sigmoid expression curves and plateau phase onsets might suggest a synchronized transcriptional regulation of IL-6 and PRDM1 expression by PIC stimulation. Further studies on the *IL-6* gene regulation by TFs or antisense RNA in the context of virally induced exacerbations in CLD are needed.

Contribution of a Disease-spanning Working Group: Lung Environment Interaction

Abstract No. 004

Short chain fatty acids do not affect inflammatory response of macrophages but might alter their proteolytic capability

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Rationale: Short chain fatty acids (SCFA) train innate immune cells and might affect asthma pathology through inflammation and remodeling. SCFA are anti-inflammatory and protective in asthma but, the underlying mechanisms remain unknown. We hypothesize SCFA affect monocyte-macrophage development (M-MD) altering the inflammatory response (IR) and proteolytic capabilities of macrophages. **Methods:** Monocytes (MC) isolated from blood by negative magnetic-activated cells sorting (Miltenyi Biotec, DE), were stimulated with granulocyte-macrophage colony-stimulating factor (GM-CSF; PreproTech, USA), sodium propionate, and sodium butyrate (both AcrosOrganics, USA). MCs were stimulated with recombinant human Interleukin (IL)-13 (Bio-Rad, USA), and polyinosinic-polycytidylic acid (Poly-IC; InvivoGen, USA). Single cell RNA sequencing (scRNA-Seq) data from the human lung cell atlas was used to identify markers of alveolar macrophages (AM). Gene expression was analyzed via quantitative polymerase chain reaction (qPCR) with statistical analysis of the mean DDCt stimulated vs. control. **Results:** Macrophage receptor with collagenous structure, cluster of differentiation (CD) 204, CD206 and CD14 were identified as markers of AM. Butyrate but not propionate decreased CD204 (-0.65±0.49, $p=0.0235$) and matrix metalloproteinase (MMP) 9 expression (-2.14±1.05, $p=0.0013$). The IR of macrophages, as assessed by IL-6 expression, towards a viral-like stimulation with Poly-IC in a pro-allergic environment (IL-13) was not affected by butyrate (1.12±2.24, $p<0.0500$). **Conclusion:** M-MD was affected by butyrate. Butyrate did not affect the IR of macrophages but decreased MMP9, suggesting a reduced matrix remodeling capacity. Further research on the underlying mechanisms of SCFA in asthma is needed.

Contribution of a Disease-spanning Working Group: Lung Environment Interaction

Abstract No. 005

Characterisation of different stem cell types by single-cell analysis of Drosophila larval trachea

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In spite of the obvious differences, the tracheal system of insects and the lungs of vertebrates have some surprising similarities. This applies to general architecture and structure, but also to organ physiology and development. The tracheal system of the fruit fly *Drosophila* is well studied for processes such as organ development and is used to analyse chronic human lung diseases such as asthma and COPD. However, there is still a lack of a comprehensive study of all of the cell types and the underlying molecular architecture. In order to fill this gap, we carried out single-cell sequencing of the entire tracheal system. We focused on characterising different stem cell types. Three groups of progenitor cells can be distinguished in the larval trachea: Spiracular Branch (SB) tracheoblasts, Air Sac Primordia (ASP) cells, and transformed epithelial cells that become precursors of the adult airway system at a specific developmental stage. The entire adult tracheal system develops from these different progenitor cells. In particular, tracheoblasts show similarities to the stem cells of the airway epithelium of the vertebrate lung and go through several stages of development towards normal epithelial cells. After remaining quiescent throughout larval life, they have the ability to proliferate, differentiate and build the entire adult respiratory system. In contrast, transformed epithelial cells have a different origin. However, they share the proliferative properties of more conventional stem cells. We hope to establish a model for progenitor cells in respiratory epithelia through detailed characterisation of these cells.

Contribution of a Disease-spanning Working Group: Single Cell Analysis

Abstract No. 006

Increased Epigenetic Aging in Nasal Epithelial Cells of Symptomatic, Allergic Asthmatics Compared to Healthy Individuals

Sonja Buchholz[1,2], Axel Künstner[3], Thomas Bahmer[4,5], Gesine Hansen[6], Erika von Mutius[7], Klaus R. Rabe[4], Anna-Maria Dittrich[6], Bianca Schaub[7], Christine Happle[6], Matthias Volkmar Kopp[8], Hauke Busch[3], Markus Weckmann[1,2]

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Background: Childhood allergic asthma is associated with allergic sensitization and altered DNA methylation (DNAm). Recent studies suggest a relationship between DNAm and age, with methylation age (mDNAge) as a biological age biomarker. However, the association between nasal epithelial cell (NEC) mDNAge and asthma/allergy remains unclear. We hypothesize that nasal mDNAge is associated with allergic asthma. **Methods:** Nasal brushes and clinical data from 120 children and adolescents aged 3-20 years were obtained as part of the All-Age-Asthma cohort. Participants were classified into three groups: asthma (n=72), wheeze (n=17), and healthy (n=31) based on diagnoses and symptom questionnaires. DNAm was quantified using the HumanMethylation850 BeadChip, and mDNAge (in methylation years, mJ) was calculated and analyzed using ANOVA, t-test and multivariate regression. **Results:** The average age was 10.9 years (asthma: 12.2 years, wheeze: 4.5 years, healthy: 11.3 years). Disease status (asthma: 31.8 mJ, wheeze: 24.8 mJ, healthy: 29.7 mJ; $p<0.001$) and atopy (with atopy: 31.4 mJ, without atopy: 28.2 mJ; $p=0.04$) were significantly associated with mDNAge (univariate). In a multivariate model ($r2=0.54$), biological age ($p<0.001$) had the greatest influence on mDNAge. Blood eosinophils ($p=0.04$) were independently associated with mDNAge. Healthy subjects with atopy had a significantly higher mDNAge than healthy subjects without atopy (with atopy: 32.7 mJ, SD 5.3; without atopy: 26.9 mJ, SD 3.4; $p <0.01$). **Discussion:** Children with allergic asthma appear epigenetically older. mDNAge increased with asthma and atopy, confirmed by blood eosinophils. Elevated mDNAge may serve as a marker for allergic asthma, but further research is needed for clinical applications of this knowledge.

Contribution of a Disease-spanning Working Group: Lung Environment Interaction

Abstract No. 007

The role of Arginase 1 therapy on myeloid-derived suppressor cells in a murine model of chronic asthma and influenza virus-induced asthma exacerbation

Tobias Brunn, Tim Lange, Charlotte Vetter, Nora Vedder, Chiel van Geffen, Philipp Gercke, Harald Renz, Saeed Kolahian

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Background: Asthma exacerbation leads to the worsening of respiratory symptoms, often induced by respiratory viral infections. Myeloid derived suppressor cells (MDSC) play a crucial role in inflammatory diseases such as asthma by suppressing the T-cell response. *Methods*: Here, we aim to investigate the efficacy of Arginase 1 (Arg-1), one of the key products of MDSCs, on airway inflammation and responsiveness in a chronic murine model of house dust mite (HDM) induced asthma and Influenza virus (IAV) H1N1 A, /Puerto Rico/8/1934 induced asthma-exacerbation. The mice were treated intraperitoneally with Arg-1, three times per week for five weeks. Lung-function, immunological, biochemical and histopathological analysis were performed three days after last HDM or IAV challenge. *Results*: We demonstrated that the number and suppressive activity of MDSCs on T cells are altered between experimental and therapy groups. I*n vivo* lung function studies showed a distinct improvement of lung function parameters in Arg-1 treated exacerbated asthmatic mice. Histopathological and immunological analyses correlated with these findings. *Conclusion:* Our results provide evidence that MDSCs and their main product Arg-1 play a crucial role in the pathogenesis of chronic asthma and IAV induced asthma exacerbation. Arg-1 treatment impacts immunological, functional, and histopathological features in the lung, improving airway inflammation and responsiveness in asthmatic and exacerbated asthmatic mice.

Keyword: Asthma, Asthma exacerbation, MDSC, Arg-1, Influenza virus, Lung inflammation

Abstract No. 008

Associations of clinical asthma phenotypes, BMI and lung function in the pediatric arm of the ALLIANCE cohort

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Background:
Asthma comprises various phenotypes, of which some are associated with high BMI. Within the ALLIANCE study, 8 phenotypes were identified by latent class (LC) analysis of symptoms and clinical features. We aimed to identify phenotypes associated with BMI and a relevant disease burden as determined by a reduced FEV1/FVC ratio (Tiffeneau index, TI).

Methods:
Clinical asthma phenotypes were defined as introduced by Hose et al. More severe phenotypes (LCs 3, 5, 6, 7, 8) were contrasted to a pool of rather benign phenotypes (LCs 1, 2, 4). Values for BMI and TI were standardized according to KiGGS and GLI population references. Associations of BMI and TI, and LCs were assessed by generalized estimated equations. Models were adjusted for study center, study group, and interaction terms with site and study group. Beta estimates (ß) are given with 95%-confidence intervals.

Results:
Data from n=200 children below and n=295 above age 6 years at recruitment were available with an average follow-up of 28,4 months. LC5 was positively associated with BMI (ß=0.74 [0.45; 1.03]; p<0.001), followed by LC6 (ß=0.36 [0.09; 0.63]; p=0.009) and LC8. BMI itself was inversely associated with TI (ß=-0,18 [-0.24; - 0,10]; p<0.001). LC5 and LC8 were inversely associated with TI (ß=-0.35 [-0.70; 0.01]; p= 0.053 and ß=-0.49 [-0.95; -0.04]; p= 0.035, respectively). Adjustment for BMI changed the estimate of LC5 and TI by 35%, whereas the association of LC8 and TI was hardly changed (8%).

Conclusion:
Though LC analysis was not informed by BMI and lung function measures, it identified a distinct asthma phenotype (LC5) associated with impaired lung function. This association was partially explained by the BMI. Nevertheless, other factors may contribute to poor lung function in this phenotype.

Abstract No. 009

Validation of flow cytometric antibody panels to determine inflammatory-driven changes in cell phenotypes of bronchoalveolar lavage

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Background: Establishing antibody panels to characterize granulocytes from bronchoalveolar lavage (BAL) using samples of healthy volunteers is challenging due to the high abundance of autofluorescent macrophages. Of particular interest, however, are phenotypical changes in eosinophils, basophils, neutrophils, and monocytes during T1- or T2-driven inflammation. Our goal was to develop and validate antibody panels that allow to quantify and characterize activation phenotypes of major leukocyte populations before and after induction of an allergic airway inflammation.
Method: Mild asthmatic volunteers underwent bronchoscopy with segmental challenge to collect BAL at baseline and 24 hours after saline or allergen challenge. BAL cells were stained with antibodies to differentiate macrophages, monocytes, neutrophils, eosinophils, basophils, and mast cells plus selective activation markers. Cell frequencies determined by flow cytometry (FCM) were verified with differential cell counts (DCC) by light microscopy.
Results: The well-known increase in eosinophils after allergen challenge was of similar magnitude using DCC (mean ± SD: 0.2 ± 0.2% to 58.5 ± 25.3%) and FCM (5.2 ± 4.6% to 52.0 ± 20.0%). Low-event cells such as basophils evading quantification by routine DCC were found quantifiable by FCM after allergen challenge with 2.4 ± 0.8 %. Intra- and inter- assay validity of cell frequencies and marker expression was high with CVs <25% for all FCM measurements.
Conclusion: The established and validated antibody panels were able to reliably distinguish cell populations in BAL that are important to characterize allergen-induced inflammatory processes. In contrast to DCC, the flow cytometric panels allowed additional phenotyping of cells by the measurement of activation markers.

Abstract No. 010

Impaired perinatal tolerance development in NLRP3-/- mice - association with regulatory B cell dysfunction

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Background: The inflammasome NLR family pyrin domain containing 3 (NLRP3) plays a crucial role in inflammasome-mediated immune responses and represents a key component of the innate immune system. The role of NLRP3 in early life tolerance development has not been investigated so far.
Objective: To assess the role of NLRP3 in early tolerance induction via mother milk in a murine model of allergic asthma-like airway disease (AAD).
Methods: During lactation WT and NLRP3$^{-/-}$ dams were tolerized by intranasal application of ovalbumin (OVA) over a course of 3.5 weeks starting 2-3 days after birth. At 5-6 weeks of age, mice received OVA in Alum or Alum only systemically and OVA in NaCl 0.9 % or NaCl 0.9 % only intranasally according to our allergic asthma disease (AAD) model.
Results: The offspring of WT animals that had been tolerized during lactation were protected from later AAD as shown by significantly reduced airway hyperresponsiveness (AHR), mucus production, pulmonary inflammation, and T_H2 cytokine production. In contrast, the offspring of tolerized NLRP3$^{-/-}$ mice developed AAD irrespective of early tolerization. WT mice but not NLRP3$^{-/-}$ mice showed a significant increase of IL-10-expressing regulatory B cells (B10 cells), increased IL-10 production in the periphery as well as increased T follicular helper (T_{FH}) cells. In contrast, Treg frequencies remained unchanged in both strains. Furthermore, expression of tolerogenic pDC was significantly decreased in tolerized NLRP3$^{-/-}$ mice when compared to respective WT mice.
Conclusion: We show that lack of the inflammasome NLRP3 hinders early life tolerance development in association with an increase of B10 cells, IL-10 production, and T_{FH} cells in WT mice, as well as a decrease of tolerogenic pDC in NLRP3$^{-/-}$ mice.

Abstract No. 011

Preconception paternal exposure to e-nicotine affects airway development in D. melanogaster offspring

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Background
E-cigarettes are increasingly used by teenagers, but it is unknown how this may affect the respiratory health of their future children. We have previously demonstrated in *D. melanogaster* that preconception maternal e-nicotine reduces body size and induces severe airway remodelling in the F1 generation. Here, we investigated the effects of paternal e-nicotine before mating affects growth and airway development in offspring.

Methods
One-day old male flies were exposed to 5-mM e-nicotine (controls: water) 8 times once per hour for 3 days in a self-constructed vaporing system. Males were then mated overnight with un-exposed females. Eggs were collected in the next 2 h to produce synchronized F1 embryos. The lengths of L1 larvae, lengths and weights of L3 larvae, and airway arborization were analyzed in F1 offspring from control and e-nicotine groups.

Results
While the length of L1 larvae did not differ between paternal exposure groups, it was strongly reduced in F1 male L3 larvae ($p<0.0001$) but not in F1 females. Although, the weights of L3 larvae remained unchanged in both sexes, ratio of weight to length of male L3 larvae was significantly reduced ($p=0.005$). In contrast to larvae, the length of adult F1 flies was significantly increased (females $p<0.0001$; males $p<0.004$). In airways, the length of the terminal cells (females $p<0.02$: males $p< 0,0001$) and number of branches (females $p= 0.05$; males= 0.03) were significantly decreased by paternal e-nicotine exposure.

Conclusion
This is the first study to show that paternal e-nicotine affects the airway development of F1 *D. melanogaster,* and this is more pronounced in male than female offspring. Future studies will examine the body size and life span of F1 adults as well as the paternal testes for altered sperm development.

Contribution of a Disease-spanning Working Group: Lung Environment Interaction

Abstract No. 012

Cigarette Smoke Exposure influences terminal cell morphology, epithelial thickness and lifespan in Drosophila melanogaster with ORMDL and SerpinB3 dysregulation

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Background Asthma development is influenced by both genetic and environmental factors. *ORMDL3* and *SerpinB3,* also termed *Scca1,* are known asthma risk genes, while early life cigarette smoke (CS) exposure is a risk factor for wheezing and asthma. We therefore asked how lifespan and airway morphology of *D. melanogaster* larvae with airway specific overexpression (OE) or knock-down (KD) of the fly homologs *dORMDL and Spn43Aa* are modified by CS exposure. **Methods** Fly lines with KD or OE of *Spn43Aa* and *dORMDL* were generated using the Gal4-UAS system. Gene-modified larvae and genetic controls were exposed to CS on 3 consecutive days (3 puffs/min for 1 h). Survival of adult flies was monitored until natural death. The structure of airways, including epithelial thickness, length of terminal cells, and their total number was analyzed by microscopy. **Results** The lifespan of adult males was significantly reduced by OE of *Scca1* and *ORMDL* and further decreased by CS. Females with OE of the genes had a shortened lifespan only after CS exposure while KD of both genes did not affect the lifespan. However, male lifespan was shortened after additional CS exposure for both. Terminal cell length varied between sexes with a significant reduction observed in females with *Scca1* OE after CS exposure. Larvae with *Scca1* OE had more terminal cells than genetic controls, which was further increased by CS. Epithelial thickness was significantly increased after *Scca1* OE and CS exposure. **Conclusion** We propose that *Scca1* and *ORMDL* are important in regulating the morphology of airways, while lifespan depends on both CS exposure and genetic factors. To further characterize the effect of *Scca1* and *ORMDL* on airway development we will now investigate the airway epithelial cell proliferation and cell integrity.

Contribution of a Disease-spanning Working Group: Lung Environment Interaction

Abstract No. 013

Specific association of extracellular vesicles-derived miRNA-17 and -106a clusters with pathomechanims of obesity-associated low type-2 asthma

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Plasma extracellular vesicles (EVs) and their cargo represent a vital source of molecular information about health and disease states and may predict specific pathomechanisms behind disease phenotypes. Here we aimed to utilize EV microRNA (miRNA) signatures to gain new insights into underlying molecular mechanisms of obesity-associated low type-2 asthma. Ten obese (OA) and ten non-obese low type-2 asthma (NOA) patients were selected from an asthma cohort alongside with ten healthy controls. Plasma EVs were isolated and characterised by nanoparticle tracking analysis, EV-associated small RNAs were extracted, sequenced and bioinformatically analysed. Based on EV miRNA expression profiles a clear distinction could be established between the three study groups by a principal component analysis. An integrative pathway analysis of potential target genes of the differentially present miRNAs revealed inflammatory cytokine (e.g., interleukin-6, transforming growth factor-beta, interferons) and metabolic factors (e.g., insulin, leptin) signalling pathways to be specifically associated with OA. The miR-17~92 and miR-106a~363 clusters were significantly enriched only in OA. These miRNA clusters exhibited discrete bivariate correlations with several key laboratory and lung function parameters. Further, plasma EVs miRNA signatures mirrored blood-derived CD4+ T-cells transcriptome data with even higher sensitivity in identifying specifically affected biological pathways. Thus, the identified plasma EV miRNA signatures and particularly the mentioned miRNA clusters were specifically linked to pathomechanims of obesity-associated low type-2 asthma.

Abstract No. 014

Intranasal EP4 agonist and Arginase-1 therapy in a murine model of asthma

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UGMLC

Background: Research findings evermore suggest a crucial role of myeloid-derived suppressor cells (MDSC) in chronic lung diseases including asthma. Previously, we showed that intravenous treatment with a prostaglandin E2 receptor 4 (EP4) agonist (L-902,688) or a pegylated human arginase-1 (BCT-100) potentiated MDSC suppressive activity and ameliorated lung inflammatory features in a murine model of asthma. *Aim:* Here, we further investigate this potential therapeutic approach by studying the local effects after intranasal (IN) application. *Method:* Using the same two-week model of house dust mite (HDM)-induced murine asthma, the effect of IN treatments on *in vivo* lung function, inflammatory features of asthma and MDSC generation and activation was studied. *Results:* Our experiments demonstrated an increased suppressive activity of pulmonary MDSCs after induction of allergic airway disease. IN treatment with both substances further augmented this effect. Additionally, treatment with BCT-100 reduced pulmonary T cells numbers. Asthmatic mice that received L-902,688 showed improved *in vivo* lung function. *Conclusion:* Our results undermine the potential of systemic or local targeting MDSCs as a future therapeutic option in airway inflammatory diseases such as asthma.

Abstract No. 015

Longitudinal analysis of blood eosinophil levels in children of the ALLIANCE cohort – mean levels and not variability are decisive for severity

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Background: Blood eosinophils (eos) are an important biomarker in patients with asthma which is used to define clinical phenotypes and make treatment decisions. While substantial variability in eosinophil levels have been shown in adults, longitudinal data in children is not available.

Methods:
We analysed children of the German ALLIANCE cohort study with at least 2 eosinophil measurements over 5 years resulting in 749 samples from 210 children with recurrent wheeze (W) and 899 samples from 236 children with asthma (A). We performed K-means clustering analysis based on mean eosinophil values and variability, defined by standard deviation (SD) and compared clinical characteristics between clusters.

Results:
Three clusters were identified. Cluster 1 had mean eos of 240/µl (SD 159-319) in W and 257/µl (202-383) in A and showed low variability. Cluster 2 showed lmean eos of (237/µl, SD 148-382 in W and 277/µl, SD 576-895 in A) but high variability. In cluster 3 mean eos were high (669/µl, SD

538-852 for W and 689/µl, SD 576-895 in A) with moderate variability. Cluster 3 was associated with rhino-conjunctivitis, polysensitization and house dust mite sensitization, the latter association was particularly strong in children with wheeze. In children with asthma, no association was seen regarding lung function, but less exacerbations/ year were seen in cluster 1. Importantly, in wheezers, cluster 1 and 2 showed higher rates of remission by age of 6 years (49% and 45% respectively) versus cluster 3 (20%), and lower rates of children with persistent symptoms / asthma (29% and 35% versus 51% in cluster 3), p=0.009.

Conclusion: Overall, variable eosinophil levels over time were seen in children and consistently high levels over time are associated with a more severe phenotype.

Contribution of a Disease-spanning Working Group: Lung Environment Interaction

Abstract No. 016

Transcriptomic characterization of type 2 innate lymphoid cells from asthma patients in response to prostaglandin D2 metabolites

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RATIONALE: Increased production of Prostaglandin D_2 (PGD_2) is linked to development and progression of asthma and allergy. PGD_2 is rapidly degraded to its metabolites, which initiate type 2 innate lymphoid cells (ILC2) migration and IL-5/IL-13 cytokine secretion in a PGD_2 receptor 2 (DP_2)-dependent manner. Inhibition of DP_2 has shown therapeutic benefit in subsets of asthma patients. Cellular mechanisms of ILC2 activity in response to PGD_2 metabolites are still unclear.
METHODS: ILC2s were isolated from peripheral blood of four patients with atopic asthma. ILC2s were stimulated with PGD_2 and four PGD_2 metabolites ($\Delta^{12}PGJ_2$, $\Delta^{12}PGD_2$, 15-deoxy$\Delta^{12,14}PGD_2$, 9α,11βPGF_2) with or without the selective DP_2 antagonist fevipiprant. Total RNA was sequenced, and differential expressed genes (DEG) were identified by DeSeq2.
RESULTS: Dynein axonemal heavy chain 1 (DNAH1) expression was significantly decreased in activated ILC2, while Layilin (LAYN) significantly increased mainly in response to $\Delta^{12}PGJ_2$. DP_2 inhibition via fevipiprant resulted in six DEG (e.g. ↓DDIT4, ↓LAYN). Hypothesis-driven data analysis revealed elevation of β_2-integrin (LFA1) and hematopoietic PGD synthase (HPGDS; exept for 9α,11βPGF_2) in stimulated ILC2, which normalized post DP_2 inhibition with fevipiprant.
CONCLUSIONS: DNAH1 is involved in recurrent respiratory infections progressing to permanent lung damage, but its role for ILC2 regulation remains unclear. Increased LFA1 and LAYN, both relevant for cell adhesion, may enhance ILC2 migration response. Elevated HPGDS would catalyze further PGD_2 synthesis and thus increase inflammation. Our results further our understanding of DP_2 initiated ILC2 activity.

Abstract No. 017

B cell activating factor of the TNF family (BAFF) in house dust mite-induced allergic asthma

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Objective: Asthma is a common multifactorial respiratory disease that is often triggered by the allergen house dust mite (HDM). The chronic disease is characterized by strengthened airway hyperreactivity (AHR), remodelling and inflammation of the airways. B cell activating factor of the TNF family (BAFF) is most prominent for its role in homeostatic development of B cells and modulated BAFF level are associated with allergic diseases. However, its role in asthma remains unclear. To better understand the impact of BAFF in allergic asthma, we used an experimental HDM-based model.
Methods: Asthma was induced by intranasal application of HDM once per week (1/w) for four weeks. Control mice received 0.9% sodium chloride only. In kinetic experiments, mice were treated 1/w for one, two or three weeks with HDM. To block BAFF in our model, anti-BAFF or isotype antibody was administered intraperitoneal. AHR was measured by invasive lung function after methacholine provocation. Cytokines and immunoglobulins (Ig) were determined by ELISA and cytometric bead array, B and T cells were characterized by flow cytometry.
Results: Compared to controls, sensitized mice showed elevated levels of BAFF in serum and bronchoalveolar lavage fluid, which increased over time in response to repeated HDM sensitizations. BAFF blockade resulted in aggravated lung function in sensitized mice, accompanied by reduced numbers of immature and mature B cell and T cell populations as well as Ig level. Importantly, BAFF blockade decreased numbers of Bregs and Tregs along with IL-10 level, whereas stimulation with recombinant BAFF protein augmented IL-10 secretion *in vitro*.
Conclusions: Our data indicate that BAFF is crucial for maintaining regulatory cell populations in HDM-induced experimental allergic asthma.

Abstract No. 018

Differential TLR8-dependent signalling in human immune cells induced by immunomodulatory archaea

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Early childhood exposure to a diverse microbial environment is inversely related to the development of asthma and allergies. Presence of different archaeal strains (e.g., *M. stadtmanae* and *M. smithii*), among others, within this microbiome or in stool samples of children has been associated with a reduced odds ratio of allergic diseases. Although both archaeal strains activate cells via their RNA and are recognized through TLR8, they possess a strikingly different capacity to induce inflammatory cytokine secretion from human immune cells. In order to get a more complete picture of their stimulatory capacity we performed an in-depth analysis of mRNA induction and cytokine release by both *M. stadtmanae* and *M. smithii* in PBMCs from healthy donors and HDM asthmatics.
As already shown by us with other cells, stimulation with *M. stadtmanae* leads to much higher activity than with *M. smithii*, drives Th1 polarization in naïve T-cells and elicits a strong Th1 and IL-10 effector response from PBMCs. However, a set of specific cytokines such as IFN-beta, MCP-1 or IP-10 are induced by *M. smithii* in comparable strength. Furthermore, the number of genes exclusively differentially expressed upon stimulation with either archaeal species varied by a factor of 6: stimulation with *M. stadtmanae* affected 309 and 294 genes after 4h and 24h, respectively, whereas stimulation with *M. smithii* affected 16 and 113 genes, respectively. In HDM asthmatics, the number of differentially expressed genes was generally smaller, in particular after the short-term stimulation (4h) with *M. smithii*.
Taken together, our results indicate that the immune response-inducing capacity of archaea is more complex than originally thought and apparently not dictated by their TLR8-dependent RNA recognition alone.

Contribution of a Disease-spanning Working Group: Microbiome-Metagenome

Abstract No. 019

Nasopharyngeal detection of 13 most common human viruses in a large cohort of children and its association with poor asthma control

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Introduction: Viral infections can trigger acute asthma exacerbations in children, but knowledge about viral presence in the upper airways and its impact on asthma severity is limited. Here we analyze the presence of pathogenic respiratory viruses in a large cohort of children and study the association of viral detection on asthma control beyond acute viral infection itself.

Methods: The study centres ARCN, BREATH, CPC-M recruited children with both established diagnoses and new-onset disease including clinical data and deep phenotyping aquisition. Written consent was obtained in concordance with the approval of the ethical committees. Between 2013 to 2016 nasopharyngeal swabs were analyzed using Multiplex-PCR (PathoFinder) which tested for 13 viruses: human rhinovirus (HRV), respiratory syncytial virus (RSV)-A, RSV-B, metapneumovirus (MPV), coronavirus (CoV)-OC43, CoV-229E, adenovirus (AV), parainfluenzae virus (PIV)-1-4, influenzae virus (IV)-A, IV-B. CT values ≤30 were considered as positive detection.

Results: Analyses of 881 nasopharyngeal swabs from 504 participants (wheezers, asthmatics and healthy controls) showed following detection frequencies: 10,4% HRV; 3,7% RSV-A; 3,6% PIV-4; 3,0% MPV; 2,7% CoVOC43; 2,2% AV; 1,9% PIV-3; 1,8% RSV-B; 1,8% PIV-2; 1,6% PIV-1; 1,1% CoV229E; 0,2% IV-B; 0,1% IV-A. RV-positive participants more often reported poor asthma control (GINA) compared to HRV-negative participants (65,9 % vs. 50,4%, chi-square, $p<0.01$).

Conclusion: The vast majority of pediatric nasopharyngeal swabs were negative for any virus. HRV was most frequently detected and significantly associated with poor asthma control. This warrants further investigations to evaluate the role of HRV "colonization" in asthma control.

Contribution of a Disease-spanning Working Group: Lung Environment Interaction

Abstract No. 020

Prediction of loss of lung function based on clinical features and wheeze trajectories

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Objectives:
To define persistence of wheeze and clinical phenotypes of asthma and to benchmark these definitions for predicting low lung function in the paediatric arm of the ALLIANCE cohort.
Methods:
Wheeze was defined as reporting any wheeze trigger or GINA night symptoms in the last 12 months. Yearly information on wheeze was entered in a latent class analysis (LCA) for classifying trajectories (n=575). In a time-to-event analysis, persistence of wheeze was given unless two consecutive periods were free from wheeze. In addition, another LCA was performed on clinical features of asthma as introduced by Hose et al 2022.
Results:
LCA for wheeze revealed a persistent, a prolonged transient, and an early transient trajectory. This classification corresponded well with the definition of persistent wheeze in the time-to-event analysis (Hazard ratio (HR)=26.5, p<0.001). The latter showed that persistence of wheeze was more common in children recruited at >=6 years and was mainly determined by atopy (itchy eyes: HR=2.3; p<0.01) and parental asthma (HR=2.1; p<0.01). In children recruited <6 years, persistence was additionally determined by duration of wheeze or cough periods (HR=1.65; p<0.01), number of hospitalizations (HR=1.44; p<0.04), and traffic proximity (HR=0.44; p<0.01). The LCA for clinical features revealed 8 distinct classes reflecting disease severity. In children >=6 years, the three severe versus the 5 milder classes predicted poor lung function (lowest quartile of FEV1/FVC) significantly (HR=3.03; p<0.01), whereas the wheeze trajectories did not predict.
Conclusions:
Classifications of clinical features such as symptom control, atopy, or hospitalization may be useful for prediction of loss of lung function, which seems to be independent from wheeze trajectories.

Abstract No. 021

Differential antibody levels to rhinovirus A/C and B in wheezing children and healthy controls

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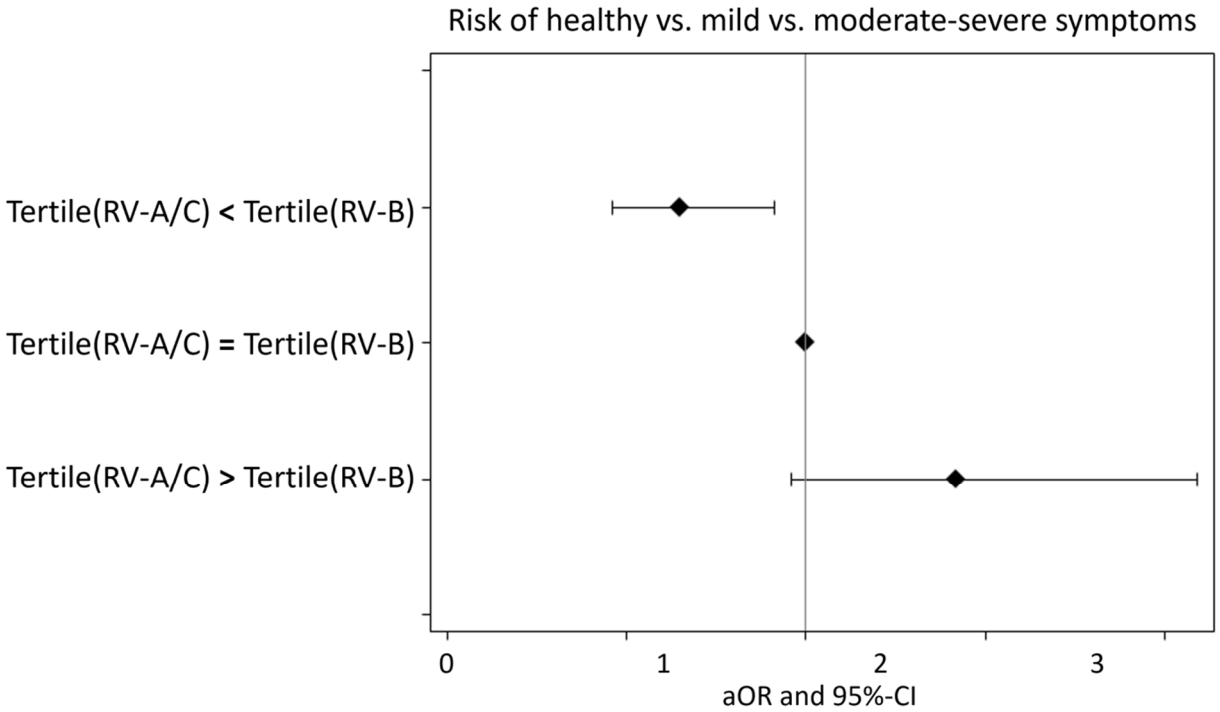
Background: We previously reported that wheezers had higher levels of rhinovirus (RV) A/C-specific antibodies as compared to healthy controls (HC). No such difference was observed for RV-B. Underlying mechanisms for this imbalance are still unknown.
Methods: Using a highly sensitive silicon chip, specific IgG antibodies to micro-arrayed N-terminal VP1 peptides representing RV-A, -B and -C species were measured in 885 serum samples from 400 children ≤9yrs (137 HC; 263 wheezers) from the ALL Age Asthma Cohort (ALLIANCE). A 3-level outcome "wheeze" was defined as healthy/mild/severe symptoms. RV-A/C and B specific IgG levels were categorized into tertiles.
Results: When including RV-A/C or RV-B in separate models, only the former showed a significant association with wheeze (OR=2.0, p<0.001 for 3rd vs. 1st tertile). However, when simultaneously including RV-A/C and RV-B, the effect of RV-A/C remained unchanged, whereas RV-B was inversely associated with wheeze (OR=0.6, p=0.024). When directly comparing the tertiles of RV-A/C and of RV-B, a trend became obvious: a decrease in wheeze was observed if RV-A/C was lower than RV-B (OR=0.65, p=0.013 compared to equal tertiles), whereas an increase if RV-A/C was higher than RV-B (OR=1.4, p=0.080):



Conclusions: A positive immune response to RV-B seems to counter-balance the risk for wheezing associated with RV-A/C. Whether this is due to specific characteristics of the RV species or due to host-specific factors needs to be elucidated.

Abstract No. 022

Activation of distinct mucosal phenotypes (nasotypes) in wheezing and asthmatic children

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Background. Allergy and asthma are defined by local type-2 immune responses. We previously showed that epithelial cells also differentiate into E1 and E2 cells *in vitro*; however, whether these subsets evolve during disease pathogenesis and other subsets exist remains elusive.

Objective. To investigate nasal phenotypes (nasotypes) based on gene expression profiling from the pediatric arm of the ALLIANCE cohort.

Methods. We analyzed the nasal transcriptome from 49 healthy, 34 wheezing, and 105 asthmatic children using whole genome microarrays. Median age was 12.5 (1.0-20.0) (healthy 12.5, wheeze 3.0, asthma 13.0). To assign the E-signatures to epithelial cell subtypes, we performed single-cell transcriptomics of primary 3D-cultures.

Results. A correlation analysis of secreted gene products confirmed antagonistic expression patterns between the E1-nasotype (*IDO*,*CSF3*,*CCL20*,*CXCL5*,*IFNG*,*OAS2*) and the E2-nasotype (*POSTN*,*CCL26*,*CST1*,*CST2,* yet unknown: *FETUB*,*CPA3*,*PRB2*,*VSTM1*). We discovered a third *IL36G*-correlated E3-nasotype (*IL36G*,*KLK7*,*KRTDAP*,*RNASE7*,*IL6*,*IL24*) and a fourth *IL10*-correlated E4-nasotype (*IL10*,*TNF*,*ICAM1*,*CST7*,*IL6*,*TGFBI*). As expected, the E2-nasotype was highly expressed in asthmatic children (age >6) and correlated with IgE, FeNO, eosinophil levels. Surprisingly, wheezing children (age 1-3) showed a clear E1/E3/E4 signature, while the E2-signature was only switched on from age 4. Single cell transcriptomics revealed that not only the E1/E2 transcriptional programs, but also E3/E4 programs were all assigned to distinct cells.

Conclusion. We show that there are four distinct nasal phenotypes in children activated in distinct patterns depending on age, type and manifestation of disease. The factors involved in their development may represent future targets for intervention.

Contribution of a Disease-spanning Working Group: Single Cell Analysis

Abstract No. 023

Development of a nitrocellulose-based microarray with infrared detection for IgE analysis

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Objective
Component-resolved allergy diagnosis is emerging, yet singleplex analyses used for specific IgE detection in clinical routine require high serum volumes and offer a limited allergen panel. We herein sought to improve our previously established multiplex dot test in terms of allergen panel, sensitivity and required sample volume.

Methods
Sixteen allergens, namely Der p 1, 2, 5, 7, 10, 13, 20, 21, 23 and Der f 1, 2, 5, 20, 21 as well as Phl p 1 and Bet v 1 were printed contactless on glass slides with 16 nitrocellulose-coated compartments with 17.5 ng of each allergen. After blocking, each compartment was incubated with 25 µl of serum. For standardization, 4 compartments on each slide were reserved for negative serum and negative serum spiked with 1, 6 and 30 kU/l of natural anti-Der p 2 each. Detection was performed with an anti-IgE antibody conjugated with a near-infrared fluorophore. The washed and dried slides were analyzed on an Odyssey CLx infrared imager at 800 nm.

Results
Compared to the multiplex dot test, the developed microarray requires only half the sample volume while providing a wider allergen panel. Further, it had superior sensitivity with no significant differences to ImmunoCAP results and a mean signal to noise ratio of 10.8 (95%CI ± 5.5, N = 12). The high linearity with a mean correlation coefficient of 0.9936 (95%CI ± 0.0075, N = 12) allows for a simplified analysis compared to the previously used logit-log normalization of the multiplex dot test.

Conclusion
The developed microarray is a highly sensitive and serum-saving assay for IgE detection combining the high protein-binding capacity of nitrocellulose with superior sensitivity of near-infrared dyes compared to UV-Vis fluorophores and hence is highly valuable when analyzing children's sera.

Abstract No. 024

Pulmonary DC subsets in experimental model of asthma and tolerance at single cell resolution

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Background: The transcription factors Irf8 and Irf4 regulate molecular programs responsible for development and functional heterogeneity of dendritic cells. Irf8 controls development of DC-progenitors towards pDCs and conventional dendritic cell subsets involved in antigen presentation to $CD8^+$ T cells whereas, Irf4 supports generation of dendritic cells that are involved in stimulation of CD4 T cells.
Objective: Understanding impact of inflammation and tolerance on conventional pulmonary dendritic cells at single cell resolution using single cell RNA sequencing.
Methods: Using murine asthma protocol applying OVA as a model antigen, we systemically sensitized mice followed by intranasal challenge with the model allergen leading towards induction of experimental asthma or tolerance. Flow cytometry based cell sorting of pulmonary dendritic cells was done followed by 10X Genomic platform based single cell generation and sequencing.
Results: Our analysis reveals existence of two Irf8-expressing conventional pulmonary DC populations/subsets based on differential expression of the transcription factor *Batf3* and the chemokine receptor *Xcr1*. The two Irf8-expressing DC subsets are found both in inflammation and tolerance model but differ in their transcriptomic patterns showing differential capabilities in induction of inflammation and tolerance.
Conclusion: We hereby provide evidence at single cell level on existence of multiple subsets of Irf8-expressing DC and, thus propose existence of two functionally different subsets within this cluster.

Contribution of a Disease-spanning Working Group: Single Cell Analysis

Abstract No. 025

R848 stimulation during pregnancy and lactation affects distribution and immunological phenotypes of pulmonary myeloid antigen presenting cells in experimental asthma

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Background: Maternal exposures during pregnancy and lactation are important modulators of immune responses. It is unknown, if maternal exposure to TLR7/8 agonists influences asthma development in the off-spring.

Methods:

To investigate transmissibility of R848 to the fetus and to the suckling pup, we used an IFN-b-reporter albino C57BL/6 females (C57BL/6 Tyrc-2J) mated with IFN-ß-Reporter-males (*C57BL/6 Tyrc-2J Ifnb1tm2.2Lien*). For *in vivo* imaging, pregnant mice were treated intra-venously on E10 and E17 or during lactation on the 7th and 14th day post littering and IVIS imaging done. To assess effect of lactational application, suckling mums were treated with R848 on days 5, 10 and 15 after delivery. Offspring of lactating mums was subjected to an ovalbumin asthma model after 5 weeks of life. Immunophenotyping of lymphatic organs and lung was done in offspring from mums treated during gestation as well as lactation.

Results:

TLR7/8 agonist R848 crosses the placental barrier during pregnancy and is also transferable via mother milk directly to the offspring as shown by inducible-expression of IFN-ß both in the E10 and E17 embryos as well as in pups on days 7 and 14 after delivery by IVIS imaging.

R848 exposure of suckling mice attenuates eosinophilic airway inflammation in the ovalbumin asthma model while thymic regulatory T cells were increased. In addition, frequencies of alveolar and interstitial macrophages were attenuated in offspring of mums exposed to R848 during lactation.

Conclusion:

TLR 7/8 agonists passes the placental and mother-milk barrier and can influence the developing immune system of the fetus or newborn mouse. R848 exposure during lactation induces changes in adaptive and innate immune cells and confers protection from asthma in OVA treated mice.

Contribution of a Disease-spanning Working Group: Lung Environment Interaction

Abstract No. 026

The role of macrophages in farm dust induced protection against allergic asthma

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Allergic asthma is a chronic disease with an especially high prevalence among children, which often prevails into adulthood. Interestingly, several independent studies have shown that growing up in a rural environment protects children from developing allergies and allergic asthma.[1]A concerted effort has been made to elucidate the underlying cause of this protective effect, but the mechanism has not been fully understood and thus requires further investigation.

We hypothesize that repeated exposure to a farm environment primes the innate immune system, specifically macrophages, thus attenuating their response towards allergens. In a murine OVA induced allergic asthma model, we could show that a repeated intranasal application of farm dust during the sensitization phase significantly reduced airway hyper responsiveness (AHR) (Rn at 24mg/ml MCh from 2.5 to 1.8), eosinophil number (90% less) in BAL fluid and OVA specific IgE in serum (112 to 9.9ng/ml). Immunohistochemistry (IHC) and fluorescence assisted cell sorting (FACS) showed an increase in macrophage numbers in the lung upon farm dust treatment. We performed scRNA sequencing of lung tissue, which revealed shifts in the prevalence of lung-resident macrophages and monocyte-derived macrophages. To investigate the effect of farm dust treatment and the underlying molecular mechanism, we carried out e*x-vivo* experiments with murine bone marrow derived macrophages (BMDMs). Repeated exposure (2x24h) to farm dust primed cells towards a reduced pro-inflammatory cytokine response upon secondary stimulation with LPS. However, the exact mechanism by which farm dust primes macrophages and their overall role in the protection against allergic asthma needs further investigation.

Contribution of a Disease-spanning Working Group: Lung Environment Interaction

References:
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Abstract No. 027

The timing of microbial colonization affects the development of the immune system

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Background
In the first years of life, the composition of the microbiome changes dynamically, which has a huge impact on the regulation of the immune system development. Further, infection with *Haemophilus influenzae* (*H. infl.*) in childhood has been related with an increased risk for developing asthma. However, it is still unclear which of the two events is responsible for the other. In the first step to unravel it, we analysed immune status of gnotobiotic mice colonized with a restricted gut oligo-mouse-microbiota (OMM12) at different time.

Methods
Immune cells in the lungs, thymus and bone marrow were screened by flow cytometry (LSRII, BD) in germ-free (GF) mice colonized with OMM12 by birth (early colonized) or early adulthood (late colonized). GF and SPF mice were used as controls. Data are expressed as percentages. Serum IgE concentrations were measured by ELISA.

Results
GF mice had notably higher serum IgE levels compared to SPF (p=0.001), early (p=0.003) and late (p=0.03) colonized groups. In the lungs, GF mice had decreased levels of CD4+T cells, innate lymphoid cells (ILC) type 2, eosinophils and granulocytes. A slight increase of T cells was observed in thymus of GF compared to early and late conlonized mice. Dendritic cells were elevated in the bone marrow of GF mice, and a certain distinction between the groups was observed regarding ILC2 and granulocytes.

Summary
The pilot data indicated that the timing of microbial colonization can cause differences in the immune status. To confirm this, late colonization will be performed at later adulthood to induce even bigger distinction between the groups. Next, immune screening between groups will be made after intranasal inoculation with *H. infl*., and the asthmatic phenotype will be assessed upon IL-33 challenge.

Contribution of a Disease-spanning Working Group: Microbiome-Metagenome

Abstract No. 028

The role of EP4 agonist therapy on myeloid-derived suppressor cells in a murine model of chronic asthma and influenza virus-induced asthma exacerbation

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Background: Previous findings of our group showed that prostaglandin E2 receptor 4 (EP4) agonism reduces airway inflammation in a murine model of acute asthma, through increasing the number and suppressive activity of MDSCs. *Method:* To investigate this further, we used a chronic murine asthma model with house dust mite (HDM) application three times / week for five weeks and an asthma exacerbation model with influenza virus (IAV) H1N1 A/Puerto Rico/8/1934 application in the last HDM challenge day. Mice were treated intraperitoneally with the selective EP4-agonist, L-902,688, over five weeks. Three days after the last HDM / IAV challenge, the *in vivo* lung function, the number and suppressive activity of MDSCs, histopathological changes in the lung and bronchoalveolar lavage cell counts were measured. *Results:* The chronic asthma and asthma exacerbation impacted functional, immunological and pathological features of the lung, partly through increased MDSCs number in lungs. EP4-agonist treatment modulated lung inflammatory and histopathological features, as well as suppressive activity of MDSCs in both chronic asthma and asthma exacerbation models. Strikingly, in asthma exacerbation, we showed improvements in lung functional parameters of EP4-agonist treated mice. Virus shedding was impacted by EP4-agonist treatment in exacerbation model. *Conclusion:* Our findings from an experimental setting suggest possibilities for asthma and asthma exacerbation therapy using EP4-agonists.

Keywords: EP4, Myeloid-derived suppressor cells, Animal model, Asthma, Asthma exacerbation

Abstract No. 029

Association of asthma severity with high sensitivity C-reactive protein (hsCRP) in the ALLIANCE study

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Background: High sensitivity C-reactive protein (hsCRP) assays can detect systemic low-grade inflammation in various inflammatory conditions. We investigated whether hsCRP levels in the serum of children with preschool wheeze (N=280) or school-age asthma (N=319) are different compared to age-matched healthy controls (HC, N=75+210) in the ALLIANCE (The ALL Age Asthma Cohort) study.
Methods: hsCRP levels were assessed by AU5800® System (Beckman Coulter), and multivariable regression models were conducted to investigate the relationship between hsCRP at study inclusion and clinical variables related to asthma, including disease control, medication, atopy, and environmental factors. Severe cases were defined by GINA control status/awakening at night and/or latent class analysis based on clinical symptoms.
Results: No direct association of hsCRP and asthma/wheeze vs controls was identified. Severe cases expressed higher hsCRP levels vs non-severe cases at all ages ($p<0.01$). Asthma medication (inhaled corticosteroid) was associated with a decrease in hsCRP (estimate-0.15, $p=0.003$), especially among children with asthma. Among wheezers aged 0-6y, hsCRP was negatively correlated with age ($r=-0.2$, $p<0.001$), while among asthmatics (12-18y), hsCRP was positively correlated with age ($r=0.3$, $p<0.001$). A positive correlation between hsCRP and BMI z-score ($r=0.4$, $p<0.001$) was observed among asthmatics but not wheezers.
Conclusion: The study provides insights into the association between hsCRP levels and clinical variables, including asthma severity in pediatric preschool wheeze and school-age asthma. Further studies are needed to explore underlying mechanisms, unravel the potential of hsCRP as a biomarker for identifying children at risk of severe asthma and monitor disease control.

Abstract No. 030

Impaired expression of genes related to innate and adaptive immunity in children with wheeze in the ALLIANCE cohort

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Background: Preschool wheezing is the first symptom of subsequent asthma. RNA sequencing (RNA-Seq) is a powerful tool for profiling gene expression, which helps to identify key regulatory signaling pathways and associated genes that may contribute to the development of childhood asthma.
Method: We analysed RNA-Seq of children with preschool wheeze (N=130) and age-matched healthy controls (HC, N=44). Whole blood was stimulated with LPS and antiCD3/CD28 (48h), RNA was extracted, and sequencing was performed with NovaSeq 6000 (2x100 bp, lanes; 30 million reads; TruSeq stranded mRNA library, Illumina). A linear regression model on expression data, with phenotypes and sex as independent variables, was applied.
Results: RNA-Seq analysis demonstrated successful activation and differential expression of genes following both stimuli, with no centre or batch effect. Both stimuli increased gene expression of the inflammatory response (e.g., *CXCL5, SERPINB2*) and tissue repair (e.g., *IL-22, COL23A1*). Gene expression in wheezers vs. HC differed by increased expression of antigen-presentation-related markers (*CD207,* antiCD3/CD28-stimulation) and response to environmental pollutants (AHRR, LPS-stimulated).
Conclusion: RNA-Seq analysis identified significant differences in gene expression profile between innate and adaptive stimulation in preschool wheezers and HC. These findings provide valuable insights into the underlying molecular mechanisms and may partly support the early identification of children with subsequent asthma.
Outlook: This cross-sectional design will now be followed by further RNA-Seq measurements (n=576 samples) to achieve a longitudinal and cell-specific data set (CD4/CD8 cells, n= 196 samples) to provide a better insight into the development of childhood asthma.

Abstract No. 031

Repetitive rhinovirus infection causes transcriptome and epigenetic changes in airway epithelium

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Background: Recurrent human rhinovirus infection in early life has been associated with asthma development. We hypothesized that repetitive HRV infection aberrantly amplifies innate immune response in airway epithelial cells.
Methods: Primary normal human bronchial epithelial cells were cultured at the air–liquid interface. They were infected with HRV-16 three times, twice or once, and then exposed to IL-13. At the end of the experiments, the levels of inflammatory mediators in cell supernatant were quantified, mRNA was sequenced at the single-cell level (scRNASeq) and global DNA methylation (DNAm) was measured.
Results: Pre-infected cells (three-times/twice/once) released significantly more inflammatory mediators including IL-4, IL-5, IFN-λ1, IL-8 and IP-10 following IL-13 exposure, in comparison with non-infected ones. This was particularly prominent in cells that were infected three times. Additionally, scRNASeq revealed 453 upregulated and 148 downregulated genes in total cells when comparing three-times HRV infection plus IL13 to IL13 alone. Out of these differentially expressed genes, decreased and increased methylation levels were detected in 322 and 276 genes, respectively. Specifically, the expression levels of IFI27, IFIT1, CXCL10, IL33 and GSDMB were significantly increased in pre-infected cells (three-times) after IL-13 treatment, compared to IL-13 alone. In line, decreased DNAm levels were detected at the promoter regions of IFI27, IFIT1 and GSDMB.
Conclusion: Recurrent HRV-16 infection upregulates secretion of type 2 cytokines in the airway epithelium. First scRNASeq analyses show upregulation of several genes related to asthma development, partly with concomitant decrease in DNAm of their promoter regions.

Abstract No. 032

Clinical impact of pandemic related changes on the epidemiology of respiratory infections

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Introduction: After lockdown periods, an increase of respiratory infections was observed worldwide. Since then, there have been several reports of an increasing burden on children's hospitals, leading to compromises in the care of acutely and chronically ill children. We aimed to characterize changed epidemiological pattern of circulating viral infections.
Methods: We assessed the number of patients with respiratory infections and the annual distribution of virus detections between 2019 and 2022 based on 4809 clinical samples of 4131 patients in a German pediatric tertiary care center. We investigated the impact of lockdown periods on circulating spectra of viruses, pattern of coinfections, age and seasonality of these infections in children.
Results: A fourfold increase in the number of virus detections was observed in 2022 vs 2019 with a doubling comparing 2021 to 2022. In contrast to previous years, constantly high rates of RV infections were present in 2022, independent of the season, while other viruses showed less changes in seasonality. SARS-CoV-2, Parainfluenza- and Metapneumovirus detections increased significantly in 2022. (2019 vs 2022 p <0,01). Coinfections with two or more viruses occurred more frequent as compared to prepandemic years, especially in very young children (2019 vs 2022 p <0,01).
Conclusion: Compared to pre-pandemic years, there has been a dramatic increase in respiratory tract infections with an incrementing spectrum of viruses and an outstanding gain in RV infections, which have led to a high rate of hospital admissions, particularly in conjunction with other viruses. This is causing acute shortages of medical care and may lead to a long-lasting burden on the health system by increasing the risk of chronic respiratory diseases such as asthma

Contribution of a Disease-spanning Working Group: Lung Environment Interaction

Abstract No. 033

The impact of viral infections on children and adults with asthma and wheeze - lessons learned from the COVID-19 pandemic

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Introduction: COVID-related measures have had a complex impact on patients with asthma and wheeze. While during lockdowns numbers of respiratory infections were low, an increase of circulating respiratory viruses was observed in the post-lockdown era. We aimed to investigate the impact of ex- and intrinsic factors on children and adults with asthma and wheeze.
Methods: Within the ALL Age Asthma Cohort (ALLIANCE), we assessed data regarding disease control, medication and etiology of exacerbations at four time points, during and after lockdown periods. Lockdown (L1): Feb-Aug 2020: n= 279, Lockdown (L2): Oct–Apr 2020/21: n= 411, Post-Lockdown (PL1) Jun-Oct 2021: n= 331 and Post-lockdown (PL2) Oct 21-Apr 22 n=270.
Results: During lockdowns (L1/L2), in preschool children (age 0-5.9 years) and by trend in children from 6 to <18 years, low proportions of uncontrolled asthma according to GINA criteria were observed, while a loss of disease control was reported in the post-lockdown period (preschooler p=0.03 and children p=0.09). The prevalence of reported respiratory infections after lockdowns was higher for preschooler (PL 64% vs. L 39%; p=0.03), and older children (24% vs. 10%; p<0.01). Multivariate, mutually adjusted models revealed, that a substantial proportion of the effect of lockdowns on better disease control especially in preschoolers was explained by the reduction of infections. Adults reported worsening of their asthma unrelated to infections, but an age dependent effect was found for exposure to mold as a predictor for worsening (>55y, p=0.0095, PL1).
Conclusion: COVID-19 related measures caused a reduction followed by a re-emergence of

respiratory virus infections, which influenced the course of disease in children with recurrent wheeze and asthma, but not in adults

Contribution of a Disease-spanning Working Group: Lung Environment Interaction

Abstract No. 034

Airway specific transcriptional analysis of ORMDL in cigarette smoke exposed Drosophila melanogaster larvae

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Background: The ORMDL3 sphingolipid biosynthesis regulator locus/GSDMB has been associated with childhood asthma onset in humans. It is also known that early life exposure to cigarette smoke (CS) is associated with a higher risk of asthma development. We therefore investigate how airway specific dysregulated ORMDL expression in *Drosophila melanogaster* larvae affects the tracheal transcriptional response in interaction with CS.
Methods: RNA-Seq (Illumina, NextSeq500) was performed in isolated trachea of 5-day old *Drosophila* larvae with overexpression or knockdown of *ORMDL* who were exposed to CS, as well as from genetic and air controls. Reads were mapped to the fly transcriptome by a pseudoalignment approach from *kallisto*(0.46.1). For the differential expression (DE) analysis, two R libraries, *edgeR*(3.38.4) and *DESeq2*(1.36.0) were used. The resulting genes from *DESeq2* were grouped into enriched gene ontology terms with *DAVID*(v2022q4).
Results: CS did not affect the airway transcriptome in wild-type flies. By contrast, flies with an additional *ORMDL* dysregulation had altered regulation in immunology (in particular lysozymes), metabolism and proteolysis processes. In gene-modified male flies the response to CS was stronger than in females (the amount of differently expressed genes was higher). *DESeq2* detected more DE genes than *edgeR*.
Outlook: We will next investigate how biological processes of interest such as e.g., sphingolipid synthesis, are influenced by ORMDL in interaction with CS. These data will then be linked to physiological and morphological data of the same fly (see abstract from B. Ehrhardt et al.).

Abstract No. 035

Bacterial lysate OM-85 showed efficient improvement in immune host response of RV1b-infected murine lung tissue slices

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Rhinovirus (RV) is a main cause of the common cold and a major risk factor for exacerbations in chronic respiratory disease. RV infections in mouse models are cleared very quickly, so only mild to moderate infections are represented. From the 3R perspective, ex vivo models such as precision-cut lung slices (PCLS) can be used to overcome this problem. OM-85 is a soluble lysate of bacteria isolated from the respiratory tract and used to prevent respiratory infections. This dataset shows the effect of OM-85 against viral infections on mouse PCLS.

PCLS were prepared from agarose-filled Balb/c lungs, treated prophylactically with OM-85, infected with RV1b and post-treated with OM-85 for up to two days. The immune changes induced by the viral infection and OM-85 treatment were analysed.

Ex vivo RV1b-infected murine PCLS induced secretion of IL-6, IP-10 and IFN-α. Pretreatment with OM-85 resulted in modulation of several immune mediators. Transcriptomic analysis confirmed a strong immunomodulatory effect of OM-85 in RV1b-infected and non-infected PCLS. Transcriptomics showed that OM-85 alone induced differentially expressed genes (DEGs) and the number of DEGs was higher in the presence of RV. Principal component analysis revealed distinct clusters indicating product- and virus-dependent as well as -independent changes induced by OM-85. Treatment and infection of PCLS revealed early points of immunomodulation that may model the immune response.

The results showed a very pronounced immunological host effect of OM-85 alone and in addition to the RV-induced immune response in murine PCLS. OM-85 could lead to priming of immune cells in 3R-compliant PCLS models and enable more efficient clearance of viral infections.

Abstract No. 036

Airway deficiency of specific microRNAs delay development in the fruit fly.

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Background
In earlier work, we had observed a deregulation of airway developmental pathways in a fly model of early-life cigarette smoke exposure. miRNAs are highly conserved epigenetic regulators of transcription that also respond to environmental factors. We therefore asked if microRNAs are involved in the smoke induced deregulation of developmental pathways.
Methods
RNA-Seq data from *Drosophila melanogaster* larval airways exposed to cigarette smoke or air were categorized into specific pathways (HIPPO, BMP and EGFR). 25 miRNAs predicted to target all three pathways (targetscan.org) were selected and transgenic lines each lacking one of the identified miRNAs were ordered. The developmental stages were monitored. The larval length (Microscope OLYMPUS BX51, cellSens Entry v1.14), the area and the number of the branches of airway terminal cells (ImageJ NeuronJ v1.44) were measured.
Results
13 lines had a stable construct and showed fluorescence in tracheal tissue as a prerequisite for further analysis. The development assays showed reduced relative numbers of pupae in fly lines deficient for miRNA-4, 9b, 9c, 10, 133, 210 and 964. Despite the developmental deficits, the L3-larval length was comparable to the wild-type control. The area and the number of branches of the terminal cells were similar among these fly lines. Concerning terminal cells, only miRNA-1006 showed a higher number of branches in females.
Conclusion & Outlook
Seven fly lines each deficient for one specific microRNA have a lower capacity for successful pupation. These fly lines were chosen to further analysis how individual miRNAs may influence physiological fitness parameters as well as airway epithelial fine structure by assessing the tracheal epithelial thickness and its cell shapes.

Contribution of a Disease-spanning Working Group: Lung Environment Interaction

Abstract No. 037

Association between urinary eosinophil-derived neurotoxin (EDN) with reduced lung function in pediatric asthma: ALLIANCE study

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Background: Eosinophil-derived neurotoxin (EDN) is a biomarker for eosinophilic activation which can be assessed using robust immunoassays in diagnostic laboratories. Urinary (u)EDN may allow non-invasive monitoring of asthma, however, clinical recommendations for uEDN are unavailable. We aimed to evaluate the utility of uEDN as a marker of disease activity in pediatric asthma.
Methods: We assessed urine samples of 371 children from the German ALLIANCE cohort: 169 preschool wheezers (<6 years), 80 asthmatics (≥6 years), and 122 healthy controls using the ImmunoCAPTM EDN Assay. Creatinine (Cr)-adjusted uEDN values were used. The data was analyzed using linear and multivariable regression (adjusted for age, sex, site), correlation and association tests, (non-) parametric statistics.
Results: uEDN/uCr values were higher in atopic vs non-atopic preschool subjects (p=0.035) and associated with the sum of allergen-specific IgE in younger (r=0.24, p=0.003), and older subjects (r=0.23, p=0.043). uEDN/uCr was marginally a good determinant for overall atopy (subjects below 6 years: p=0.078, and atleast age 6 years: p=0.058). uEDN/uCr values were higher in T2-high vs T2-low cases (p<0.001). Similar proportions of subjects were observed to belong to a specific T2 asthma phenotype (using uEDN/uCr or blood eosinophils – combined to allergen sIgE). Among asthmatics, there was a significant correlation of uEDN/uCr with a reduction in lung function (FEV1

z-scores: r=-0.30, p=0.007, and FEV1/FVC z-scores: r=-0.24, p=0.038). uEDN/uCr was an independent determinant of FEV1 (p=0.038), and FEV1/FVC (p=0.080).
Conclusion: Our data shows that uEDN/uCr may serve as a non-invasive biomarker for lung function in asthmatic children – showcasing the potential of of uEDN/uCr as a biomarker.

Contribution of a Disease-spanning Working Group: Lung Environment Interaction

Abstract No. 038

Intravital imaging of mucus in the trachea indicates the effect of mucolytic treatment in asthmatic mice

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Asthma can lead to excessive mucus production resulting in severe symptoms and increased mortality. In asthmatic mice, treatment with isotonic saline (IS) and hypertonic saline (HS) lead to mobilization of mucus. However, both IS and HS induced transport of large mucus chunks that potentially obstruct airways. In this study, we investigated if the reducing agent Tris(2-carboxyethyl)phosphine (TCEP) can dissolve mucus chunks in asthmatic mice.
To induce asthma symptoms, IL-13 was applied, and microscopic optical coherence tomography (mOCT) was used to image mucus transport through the intact trachea of freely breathing mice. ATP-γ-S was used to stimulate mucus secretion and IS and HS were used to mobilize mucus. TCEP was given prior or after application of IS or HS. Mucus in the lungs was quantified in AB-PAS-stained sections.
Mucus secreted after ATP-γ-S nebulization was transported via the trachea after mobilization through IS or HS. TCEP administration led to extinction of mucus chunks. Likewise, TCEP pretreatment prevented the thickening of the mucus layer in the trachea by IS or HS administration. Histologic analysis revealed mucus in the airways after ATP-γ-S stimulation. After TCEP administration, hardly any mucus was detectable in the lungs independently of HS or IS application. Administration of TECP on explanted mouse tracheae in concentrations used *in vivo* impaired ciliary beating indicating possible impairment of intravital mucus transport. Since measuring mucus transport by mOCT relies on chunks of mucus being transported, we are currently unable to assess if TCEP impairs mucus transport *in vivo*.
Our results indicate that TCEP effectively dissolves mucus chunks in IL-13 treated mice. Whether TCEP impairs mucus transport *in vivo* remains to be determined.

Abstract No. 039

Seasonal dependence of symptom control in wheezers and asthmatic children of the ALLIANCE cohort

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In ALLIANCE, study participants are recruited at different time points over the year. Previously, we found that serum cytokines of ALLIANCE participants show a specific patterns of variability over time. Now, we wanted to assess whether GINA control status is also associated with seasonal time point of inclusion so that season should be considered as a critical confounder.

Method: We used a cosinor model to evaluate seasonal effects on the GINA control status of asthmatic children at baseline visit (BL) and the 12 months follow up visit (12FU) (n= 304 at BL; n=271 at 12FU) and children under the age of 6 years with recurrent wheezing (n=314 at BL and n=242 at 12FU). To control for different seasonalities within Germany, site was included in the model as a covariable.

Results: We found no seasonal effect on the GINA control status in asthmatics during BL and 12FU. Analyzing single items, we found a seasonal effect on the lung function in asthmatics at BL and on symptoms at night during the 12FU. Lung function was worse and symptoms at night were more frequent during the winter months.
Since there is no standardized tool to evaluate symptom control in children under the age of 6, we used the GINA score in our wheezing cohort. In wheezers, we found a seasonal effect on symptom control during BL but not at 12FU. The majority of wheezers had no symptoms in month June. As in asthmatics, we found a seasonal effect on symptoms at night during BL and 12FU. Additionally, use of rescue medication showed a seasonal effect in wheezers at BL with peak usage in December.

Conclusion: The seasonal effects found in wheezing children are probably due to more frequent infections during the winter months. Seasonality should be considered as a covariable in any analysis using this cohort.

Abstract No. 040

Comprehensive genome analysis of a pollen associated Pantoea agglomerans isolate and response to Nitrogen oxides (NOx) exposure

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Pollinosis is a significant public health burden worldwide, whose prevalence keeps increasing rapidly. The ability of pollen allergens to induce allergic sensitization is highly dependent on the co-delivery of non-allergenic adjuvant compounds like bacterial lipopolysaccharides (LPS). However, it is unclear whether frequent NOx exposure enhances the adjuvant activity of pollen-associated microorganisms towards a pro-allergenic Th2 skew by altering LPS structure and thus influencing the biological activity.
We explored putative virulence factors of *Pantoea agglomerans* 11-6, a member of the microbiome of mugwort pollen. Genome analysis revealed the presence of several virulence factors such as proteases, T6SS effectors and hemolysin that might contribute to allergic sensitization by inducing innate immune responses favoring Th2 differentiation or even directly by facilitating allergen uptake and DC maturation. With the exception of two T6SS effectors and three genes involved in LPS/EPS biosynthesis, virulence factors are highly conserved among *Pantoea agglomerans* strains.
Further we studied transcriptomic responses of our isolate to NOx exposure. A transcriptomic analysis of NOx treated *P. agglomerans* 11-6 showed the induction of envelope stress response pathways mediated by *RpoE,* which induced changes in the expression levels of genes involved in LPS, EPS and biofilm biosynthesis. NOx also negatively affected ribosome assembly and amino acid metabolism. Overall, these findings suggest that air pollution might lead to alterations in cell envelope structures, which favor Th2 polarization over allergy protective Th1 immune responses.

Abstract No. 041

17Q21-variant-associated mucosal nasotypes in wheezing and asthmatic children

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The 17q21 "wheezing" locus is the best validated genetic risk factor for childhood-onset asthma. Therapies addressing the increased susceptibility to viral infections associated with the 17q21-mediated clinical phenotype represent an unmet clinical need.
Nasal brushes from 188 children (49 healthy, 34 preschool wheezers, 105 asthmatics) of the ALLIANCE cohort were subjected to a genome-wide transcriptome analysis regarding their 17q21-genotype to elucidated epithelial mechanisms in a hypothesis free manner. Nasal lining fluids were sampled.
Just one allele in wheezing children carrying variants for the 17q21 SNP *rs72163891* was sufficient to induce genotype- and asthma/wheeze phenotype-dependent increased mucosal expression of the 17q21-encoded gene *GSDMB*. Increased *GSDMB* expression was associated with an increased expression of a type-1 pro-inflammatory, cell-lytic immune- and NK-genes including key genes of an IFN-type-II-signature (*IFNG, IDO1, FASLG*), as opposed to a reduction of IFN-type-I and type-III gene expression signatures at mRNA and protein level.We demonstrate for the first time a novel 17q21-induced mechanism lead by increased 17q21-encoded mucosal *GSDMB* expression that is triggering a cell-lytic immune response along with a decrease in airway immune competence. We proposes that the excessive airway cell death and the unbalanced perturbations in the mucosal IFN signature cause the increased susceptibility of 17q21 risk allele carriers to respiratory viral infections in early life. This mechanisms forms the basis for novel interventions that may prevent the IFN inbalance and rescue the viral defence mechanisms of the airways.

Abstract No. 042

Early life dysbiosis increases airway hyperresponsiveness in mice

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Epidemiological studies in humans correlate perinatal dysbiosis with increased long-term asthma severity. However, these studies suffer from high variability of dysbiotic causes (e.g., birth mode, breastfeeding, antibiotic treatment (AbT)). Further, the time window of these factors influencing asthma remains undefined. Given that maternal antibiotic exposure in mice increases the risk of newborn bacterial pneumonia in offspring, we hypothesized that early life dysbiosis stimulates long-term immunological effects in the offspring leading to an aggravated asthma phenotype. To test this hypothesis, pregnant and nursing dams underwent AbT from embryonic day 15 until postnatal day 28 (E15-P28) at two different institutions. At six weeks of age, experimental allergic asthma (AA) was induced in offspring by four weekly repeated applications of house dust mite extract. 72 h after the last immunization, airway hyperreactivity (AHR) and pulmonary cell composition were assessed. AbT aggravated AA as characterized by elevated AHR and an increased infiltration of eosinophils to the airways. Results were similar in Lübeck and Cincinnati excluding a site-specific microbial impact. To further refine the developmental window wherein AbT influences the AA phenotype, we reduced maternal AbT to E15-P14. Surprisingly, in both institutions, E15-P14 AbT-exposed litters did not display altered AA development compared to controls. Combining these data with our previous data demonstrating that AbT from P10-P20 augments AA, this study indicates that pre- and perinatal AbT is less critical for later-in-life asthma development. This model will allow further investigation into the underlying immunological mechanisms during the more decisive later postnatal timeframe in future studies.

Contribution of a Disease-spanning Working Group: Lung Environment Interaction

Abstract No. 043

SERPIN dysregulation in asthma and COPD: old friends reinvented?

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Serine protease inhibitors (SERPINs) represent a diverse family of structurally similar proteins that are found in a wide range of organisms and cellular locations. They control proteolytic cascades in a variety of biological processes, e.g., coagulation and inflammation. SERPIN malfunctions, so called “serpinopathies”, can result in severe clinical symptoms that manifest in different organs. Some SERPINs gained attention in cancer research and our data apart from this abstract shows a precise picture of an altered SERPIN expression in lung cancer. Until now, not much is known about the role of SERPINs in non-malignant lung diseases. One of the few well studied examples is the alpha-1 antitrypsin deficiency caused by mutations in the SERPINA1 gene and a known risk factor for COPD. Beyond this, the role of SERPINs in the development and progression of COPD and especially asthma is still an open research field. The purpose of this study is to capture a SERPIN expression profile in patients suffering from COPD or asthma and compare it to healthy controls. For that, we analyze expression data generated from human airway cells. The overall data show a distinct regulation pattern for several SERPINs in COPD patients, compared to asthmatics and healthy controls. Asthmatics show a much broader variation, presumably reflecting that asthma is a very diverse disease. Therefore, we aim to match the expression profiles to different asthma phenotypes. Further, we will go beyond the descriptive data and focus on the mechanistical consequences of an altered SERPIN regulation for malignant and non-malignant lung diseases to identify if they affect disease development and/or progression and ascertain new clinical targets.

Abstract No. 044

The mechanistic role of farm dust leads to protection against allergic asthma

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Allergic asthma is an allergic disease characterized by chronic airway inflammation and airway hyperresponsiveness (Cookson, 1999). In recent years, the prevalence of allergic asthma has been increasing, especially in developed countries with high hygiene standards, while children from rural areas in Europe and other countries, especially in farming environments with frequent exposure to microbial components, are protected (Von Mutius & Vercelli, 2010), while the detailed mechanism is unknown.

In order to explore the underlying mechanism, we established the OVA-induced allergic asthma model, in which cow dust inhalation can prevent the development of allergic asthma. We showed that continuous intranasal application of cow dust could reduce airway resistance, IgE and IgG1 level in serum, the number of eosinophils both in BAL cells and lung tissues, and type 2 cytokines level (IL4, IL13, IL5, etc).

Using this model, we were surprised to find that inhalation of cow dust can lead to an increase in lung macrophages in the mouse model, by immunohistochemistry staining, and which probably provides insight into deciphering the mechanistic role of farm dust in its protection against allergic asthma. We hypothesized that cow dust provides protection against allergic asthma by inducing macrophages, which dampened the th2 cells proliferation, then reduced type 2 inflammation in the murine model. In the macrophages-T cells co-culture system, we could see macrophages dampened the proliferation of T cells, which was also confirmed by the in vivo experiment. In the future, the macrophage depletion experiment and macrophage transfer experiment will be employed to explore the underlying mechanism.

Contribution of a Disease-spanning Working Group: Lung Environment Interaction

Abstract No. 045

Linking maternal and early-life obesity to onset and clinical manifestation of asthmatic phenotypes in childhood

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Introduction: Obesity often affects the clinical course of asthma. Maternal obesity is a risk factor for obesity in children, but the impact on asthma-phenotypes remains unclear. We investigated the influence of maternal obesity and childhood obesity on the onset, severity and triggers of asthma symptoms in children.

Methods: 221 patients with diagnosed asthma or wheeze and 33 healthy controls from two sites of the pediatric arm of the longitudinal All-Age Asthma Cohort (ALLIANCE) study were investigated. Maternal (weight and BMI during pregnancy) as well as children's parameters [birthweight; BMI at Baseline Visit and Body-Fat Fraction (BFF)] were correlated with FEV_1 at Baseline Visit, GINA symptom control, hospital admissions per year, onset of symptoms and triggers for wheezing.

Results: Maternal BMI at the beginning of pregnancy was higher in the asthma cohort than in healthy controls (Mean: 24,68 kg/m^2 vs. 22,30 kg/m^2). Furthermore, maternal BMI was higher in children with increased asthma-related hospital treatments per year. In addition, birthweight was higher in children who developed wheezing in the first 6 months of life (3544g vs. 3375g). Moreover, higher BFF at baseline visit was linked to earlier onset of symptoms in the first 6 months of life (27,78% vs. 21,90%) and associated with worse GINA symptom control. Finally, higher BMI in patients was associated with specific wheezing triggers e.g. physical activity, pollen and dust exposition.

Conclusion: Our results highlight the impact of perinatal obesity on asthma triggers, onset and severity and could be used in future to identify patients at increased risk for more severe asthma. Our data raises the question, if a reduction of BMI before pregnancy could contribute to prevention of asthma in these children.

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Abstract No. 101

Mitochondrial T cell reprogramming in virus-induced COPD exacerbation and emphysema progression

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T cell dysfunction may contribute to increased susceptibility to infections and disease progression in patients with COPD. Viral infections can trigger systemic inflammation and oxidative stress, which could further damage lung tissue and contribute to COPD exacerbations. Mitochondrial reactive oxygen species (mtROS) play an important role in immune function and increased mtROS levels may promote COPD. Cytochrome c oxidase subunit 4 isoform 2 (Cox4i2) regulates ROS production at complex III and thus may affect T cell function during virus-induced exacerbations. Mitochondrial respiration and T cell characteristics were analysed in pulmonary or spleen T cells isolated from wild type (WT) and Cox4i2$^{-/-}$ mice after 2 weeks of *in vivo* cigarette smoke exposure with or without H1N1 influenza (PR8) infection, by respirometry and flow cytometry, respectively. Similarly, *in vitro*, mitochondrial respiration and ROS production were determined in activated spleen T cells

Upon infection and exposure to smoke, the Cox4i2$^{-/-}$ mice exhibited a significantly lower amount of CD8+ T cells and albumin leakage in the lung compared to WT mice. Moreover, spleen T cells from the Cox4i2$^{-/-}$ infected mice showed a similar decrease in mitochondrial respiration and glycolysis. Accordingly, *in vitro* experiments revealed that spleen Cox4i2$^{-/-}$ T cells have reduced levels of ROS release, glycolysis and IFNγ release following activation, compared to WT T cells Cox4i2$^{-/-}$ mice show less accumulation of CD8+ T cells in the virus-infected lungs after two weeks of smoke exposure, and a lack of increase in respiration and ROS levels after *in vitro* activation. Thus, Cox4i2-dependent ROS release may regulate T cell activation and differentiation during exacerbations and may affect the progression of emphysema.

Contribution of a Disease-spanning Working Group: Lung Environment Interaction

Abstract No. 102

The role of cholesterol on the differentiation of airway epithelial cells

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Cholesterol (CHOL) is an essential component present in the plasma membrane and plays an important role in the maintenance of cellular permeability and ion channel function in the airway. Several studies have reported altered CHOL metabolism leading to increased accumulation of CHOL in the airways of COPD and cystic fibrosis patients, but the impact of CHOL on the airway epithelium remains unclear. Unbiased proteomic analysis from differentiating primary human bronchial epithelial cells (phBECs) at the air-liquid interface (ALI) revealed an overall inhibition of the CHOL biosynthesis pathway during the differentiation phase. Therefore, we hypothesized that CHOL is important in the differentiation of airway epithelial basal cells into the fully functional bronchial epithelium. phBECs were differentiated under the influence of CHOL at a concentration of 80 µM via the basolateral compartment for 21 days. During chronic CHOL exposure, the cell type-specific markers were determined using qRT-PCR and immunofluorescent (IF) stainings, and the epithelial barrier integrity was measured via Transepithelial electrical resistance (TEER) measurements. Post chronic CHOL exposure, a statistically significant increase in the club cell population was observed in comparison to time-matched vehicle control. However, chronic CHOL exposure did not affect the epithelial barrier integrity measured via TEER during the differentiation phase. In conclusion, our study suggests that CHOL is a potential regulator of bronchial epithelial cell fate. In near future, we would like to understand the susceptibility of phBECs chronically exposed to CHOL to an additional injury and monitor the subsequent regeneration phase.

Abstract No. 103

A novel anti-inflammatory in COPD by blocking the activity of immune arginine methylation

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This abstract is under embargo due to IP-relevant content

Abstract No. 104

Compartment-specific collagen type IV (COL4) α-chain alterations could be associated with different COPD phenotypes

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Background: COPD is a heterogeneous disease involving airway, parenchyma and vasculature. Dysregulation in ECM composition, including basement membrane proteins, is associated with disease progression. Alterations in COL4, a major component of BM, and the precise distribution of its distinct α-chains remains relatively understudied. Hence, the identification of distribution and distinct expression of different COL4-chains in lung compartments of COPD patients could help with the phenotypic stratification

Methods: Multiplex immunofluorescence (mIF) combined with cell-type specific markers was performed for compartmental analysis of COL4α-chains. We also analyzed publicly available scRNAseq profiles (GSE136831) from donor and COPD lungs as well as from 6-months smoked murine model (GSE151674)

Results: We observed differential distribution of COL4 α-chains in various lung compartments in donors. This abundance was different as compared with COPD. Similarly, scRNAseq revealed cell-type specific enrichment patterns of α-chains. The alveolar region was enriched in COL4α2,3,4 whereas the bronchiolar region was enriched in α2 to α6-chains. In the endothelial cells, the expression of COL4α1 & 2 was prominent. A significant difference in the expression of α3,4,&6-chains was observed between donor and COPD. The lungs of air- and CS-mice showed COL4α1 to α4-chain expression, these chains were slightly increased in the CS-model. Also, COL4α1 to 4 were prominently expressed in the vasculature and parenchyma. COL4α5 expression detected in the bronchiolar region was decreased in the CS-model

Conclusion: We identified a divergent COL4 α-chain expression in the parenchymal, bronchial and vascular compartment in COPD lungs, which could be used in the future to define different COPD phenotype

Contribution of a Disease-spanning Working Group: Lung Environment Interaction

Abstract No. 105

Proteasome function as a biomarker for severe COPD endotypes

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BACKGROUND: The proteasome, the main enzymatic complex for degradation of intracellular proteins, is emerging as a potential biomarker for chronic inflammatory diseases. Its activity is impaired by cigarette smoke and in lung tissue of COPD patients. Population-based studies focusing on proteasome profiling from peripheral blood mononuclear cells (PBMC) for stratifying COPD severity and progression are missing.

OBJECTIVE: We profiled proteasome activity and expression of COSYCONET patients and validate proteasome function as a biomarker for COPD severity and endotypes.

METHODS: PBMCs were obtained from 378 COSYCONET patients. Activity-based probes were used to quantify total proteasome activity and the six distinct standard (β_1, β_2, β_5) and immunoproteasome (LMP2, MECL1, LMP7) activities. Relative RNA expression of multiple proteasome subunits (PSMA3, PSMB[5-10], PSMC3, PSMD11, PSME1) was determined by RTqPCR. Linear regression was used to analyse the association of COPD stages and BODE index with proteasome function.

RESULTS: The activity ratio of the catalytic subunits LMP7 and β_5 significantly decreased with increasing BODE index among females (%-difference -48.5% (95%-CI -70.4; -10.1) for BODE index 1-2 and -46.5% (-70.0; -4.8) for BODE index ≥3 compared to BODE index 0) but not among males. These changes in activity levels were not accompanied by significantly altered expression levels of the subunits PSMB5 (encoding β_5) and PSMB8 (encoding LMP7).

CONCLUSION: Non-transcriptional mechanisms are associated with the downregulation of the

proteasome activity in COPD. Further proteasome function analyses of subjects of the KORA cohort are in preparation to provide insight in proteasome activity and expression patterns among COPD patients compared to lung healthy controls.

Abstract No. 106

Impact of heated tobacco products, e-cigarettes, and combustible cigarettes on small airways and arterial stiffness

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Introduction: Several nicotine delivery devices are discussed as a harm reduction strategy for smoking, thereunder e-cigarettes and heated tobacco products, which are also intensively promoted by tobacco companies We investigated the acute effects of heated tobacco products (IQOS) compared head-to-head to e-Cigarettes (eDIPSE) with and without nicotine and combustible cigarettes on small airway and vascular function.
Methods: This single-center four-arm cross-overstudy included 20 healthy occasional smokers (25-5±0.7 years). The participants had to consume each device after an abstinence of 48 hours of smoking. We measured vascular function (Mobil-O-Graph™) every 5 minutes and resistance of the small airways (tremoFloÒ c-100) 5, 15, 30 and 60 minutes after inhalation.
Results: All 4 devices significantly increased the small airway resistance (R5-20 - $p<0.05$) after inhalation, whereby the significant increase in the e-cigarette without nicotine could only be measured later than in the other devices. All 4 products significantly increased parameters of peripheral and central hemodynamic and arterial stiffness (AIx@75 - $p<0.05$).
Conclusion: Heated tobacco products and e-Cigarettes induce vascular and small airway dysfunction, which indicates an increased cardiovascular risk and airway damage with no harm reduction compared to combustible cigarettes.

Abstract No. 107

Role of monocyte-related chemokines in LPS induced and COPD-related lung inflammation

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RATIONALE: CCL2, a monocyte chemoattractant, is increased in sputum of COPD patients, while concentrations of other chemokines and their roles in monocyte recruitment are unclear. Inhibition of monocyte-related chemokines might prevent monocyte recruitment into the lung and thus reduce inflammation. The main objectives were assessment of monocyte migration towards bronchoalveolar lavage (BAL) fluid samples and inhibition of monocyte migration towards BAL fluid by CCL2 and CCL3 blockade.
METHODS: Migration of monocytes, isolated from peripheral blood of healthy subjects, towards BAL fluid collected post saline or LPS challenge (n=5) was evaluated using 5 µm pore transwell plates. Furthermore, inhibition of monocyte migration towards BAL fluid collected post LPS challenge by 5000 ng/mL anti-CCL2 mAB or 2000 ng/mL anti-CCL3 mAB (n=5) was analyzed.
RESULTS: Monocyte migration was significantly increased towards BAL fluid collected post LPS compared to saline challenge ($p = 0.0065$). Induced monocyte migration towards BAL fluid collected post LPS challenge was significantly inhibited by anti-CCL2 mAB ($p = 0.0020$) as well as by anti-CCL3 mAB ($p = 0.0138$) compared to isotype control.
CONCLUSIONS: In line with chemoattractant concentrations in BAL fluid in the LPS challenge model, monocyte migration towards BAL fluid collected post LPS challenge was significantly increased compared to saline challenge. Using BAL fluid post LPS for *in vitro* migration experiments, monocyte migration towards BAL fluid was significantly inhibited by CCL2 and CCL3 antagonists. Blockade of CCL2 or CCL3 may be potential anti-inflammatory mechanisms to reduce monocyte infiltration into the lung.

Abstract No. 108

Maladaptive Immune Responses Triggered by Western Diet Increases Susceptibility to COPD

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Modern lifestyle and health inequity encompassing western style dietary patterns increase the vulnerability to a variety of chronic diseases. There is clear epidemiologic evidence that these developments are a major risk factor for chronic obstructive pulmonary disease (COPD). Crucially, recent studies have suggested that maladaptive trained immunity , a state of heightened responsiveness of innate immune cells, which is based on epigenetic innate immune memory following prior inflammtory challenge, may play a role in inflammatory disease comorbidity. We hypothesize that dietry induced changes to innate immune cell function and metabolism "maladaptive training" increases susceptibility to COPD.
Exposure of mice to high fat diet for 4 months demonstrated changes to lung architecture reminiscent of pre-COPD features, mild alveolar destruction accompanied by small airway remodelling, characterised by collagen deposition and the early development of iBALT, accompanied by CD11b+ inflammatory macrophage influx. Crucially, primary bone marrow from wild-type mice exposed it to a variety of western dietary components including ox-LDL, cholesterol and unsaturated/saturated fatty acid for 24h in culture, before mCSF maturation to macraphages, induced a much stronger expression of the pro-inflammatory cytokines IL1α, IL1β and IL6 following secondary LPS stimulation of the mature macrophages, compared to bone marrow not exposed to the dietry factors. Taken all together, our findings offer direct evidence that exposure to dietary components of myeloid cells is a contributing factor to trained immunity and potential vulnerabilities to COPD development.

Abstract No. 109

In vitro and in vivo airway epithelial thickening after cigarette smoke exposure during puberty

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Introduction
Cigarette smoking (CS) is associated with airway diseases as COPD and asthma, and is of concern in younger populations with increasing adolescence-onset smoking. The underlying mechanisms are scarcely understood. One influencing factor is oxidative stress that causes molecular and structural alterations in the lung. In this study, the susceptibility window of puberty is of interest to investigate CS-exposure and long-term effects on the adult airway epithelium.

Methods
Normal human bronchial epithelial (NHBE) cells were differentiated (Air-liquid-interface; ALI) and exposed by day 14 to mainstream CS (3R4F, University of Kentucky) or room air (air) (9 min/day) for 14 days (P.R.I.T. ExpoCube), and processed for PAS staining. 3-week-old male C57BL/6 mice were exposed to air or a low dose of mainstream CS (1 puff/min, 3R4F) for 2 weeks, followed by heavy smoking (4 puffs/min) for 4 weeks (1h once per day 5 days/week; inExpose System). At 9 weeks of age, lungs were collected for qRT-PCR, H&E and PAS staining, immunohistochemistry and airway measurement.

Results
Mucus production of NHBE ALI cultures was elevated after CS exposure and the epithelial thickness of cultures was increased in comparison to air-exposed NHBE cells. CS-exposure during puberty of male mice led to an increased bronchial epithelial thickness in adulthood. qRT-PCR revealed that CS downregulated levels of Sod2 and Nox4 in lung tissue whereas in the bronchial and bronchiolar epithelium SOD2 was increased.

Conclusion
In male mice, CS exposure during the susceptibility window of puberty led to a thickening of the bronchial epithelium in early adulthood. To clarify if reactive oxygen species play a role in these changes needs further investigation.

Contribution of a Disease-spanning Working Group: Lung Environment Interaction

Abstract No. 110

Parental smoking is associated with obstructive lung disease of prematurity independently of high genetic risk at the age of 5 years

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Introduction: Loss of lung function starts early in life and is determined by environmental and individual risk factors where epigenetic and genetic factors are of specific importance. We recently showed that a polygenic score (PGS) developed in adults and predictive for the development of chronic obstructive lung disease (COPD, Moll et al., 2020) is also predictive for lung function in premature, very-low-birth-weight (VLBW) children at age 5.8y. In this study, we investigate whether COPD-PGS outcome is associated with environmental factors such as parental smoking (PS).
Methods: Premature infants with VLBW (<1500g) were recruited via the German Neonatal Network. Genotyping (Illumina) from buccal swaps at birth were combined with spirometry and questionnaire data at 5 year follow-up. PGS was calculated according to Moll et al., 2020. For exposures, COPD-PGS deciles were used as continuous predictors in linear and logistic regression models. PS was used in t-tests/Fisher's exact test as independent variable for pulmonary function outcome (spirometry, obstructive bronchitis (OB) within past 12 mo).
Results: PS was reported in 774/1957 children at 5.8y and COPD-PGS was associated with PS-frequency (OR 1.05/decile, 95% CI 1.02-1.09). While PS was associated with lower FEV1 z-scores and subnormal FEV1 and FEV1/FVC (8.9% vs. 5.8% no smoking) at age 5.8y, there was no association between rates of OB at 5.8y and PS. The association of the COPD-PGS with FEV1 and FEV1/FVC persisted regardless of parental smoking.
Conclusion: Rates of PS in a cohort of VLBW preterm infants were high and associated with lower FEV1 and subnormal FEV1 and FEV1/FVC, independently of COPD-PGS. Environmental exposures such as PS pose additional risk for early lung function decline in those risk groups.

Contribution of a Disease-spanning Working Group: Lung Environment Interaction

Abstract No. 111

Women pass the glass ceiling in Interventional Pulmonology

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Objective: Despite the growing proportion of female physicians in medicine, they still appear underrepresented in interventional fields and leading positions. No data are available until now regarding the gender distribution in interventional pulmonology.
Methods: An online survey was sent to pulmonary physicians internationally between July and December 2022. The survey included questions on gender diversity in the pulmonology departments, proportions of male, female and gender diverse physicians performing bronchoscopy, career progression and social life.
Results: Responses were analysed from 92 physicians (mean age 45±10 years) from 47 hospitals in 17 countries, of whom 52% were women. 79% of the respondents were pulmonologists and 83% perform bronchoscopy. Although men still dominate bronchoscopy (65% vs. 43%) and are more likely to be involved in research (89% vs. 77%), the observed difference is statistically not significant (p=0.135 and p=0.281). Leading positions are held by 60% of male respondents vs. 23% of female respondents (p=0.002). Also more men are reported to have academic awards. Discrimination due to gender was reported from 5.3% of all men and 26.8% of all women (p=0.023).
Conclusion: Men and women are nearly equally engaged in interventional pulmonology. Leading positions and academic awards are still hold from men. The way through the glass ceiling seems to open up for women in interventional pulmonology.

Abstract No. 112

Chromatin dynamics via HMGN5 regulates COPD susceptibility

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A role for epigenetics in COPD susceptibility is emerging. We previously detected spontaneous emphysema development in HMGN5 deficient mice. HMGN5 binds to the nucleosomal core particle of chromatin, competing with histone H1, thus altering chromatin structure and function. Here we investigate the contribution of HMGN5 to the development of emphysema. COPD patient lungs were analyzed by RNAseq and immunofluorescence. B6 and Hmgn5-/- mice were treated with porcine pancreatic elastase (PPE, 40U/Kg) and analyzed on d28. siRNA knock-down of Hmgn5 in ATII cells (A549) was followed by wound healing, apoptosis and cell proliferation assays.RNAseq of COPD patients demonstrated a 58% reduction ($p<0.01$) in HMGN5 expression in the lungs compared to healthy individuals. Furthermore, immunofluorescence analysis revealed HMGN5 is located in bronchial epithelial cells and ATII cells in humans and mice. B6 mice exposed to PPE demonstrated a 32% ($p<0.05$) reduction in Hmgn5 expression at d28. In addition, HMGN5 deficient mice had greater impaired lung function compared to wild type controls following PPE exposure (Dynamic compliance 0.084±0.007 v 0.069±0.006ml/cmH2O, $p<0.001$) with greater evidence of emphysema (MCL 54.75±6.09 v 40.40±5.09µm, $p<0.01$) and increased apoptotic ATII cells. Mechanistically, in vitro studies revealed that downregulation of HMGN5 with siRNA impaired the wound healing ability of A549 cells and increased the number of apoptotic cells with a concomitant decrease in their proliferation rate. Single cell data suggests a role of HMGN5 in regulating cell cycle genes leading to the deregulated proliferation rates.This data suggests a novel causal link between chromatin dynamics and emphysema development.

Contribution of a Disease-spanning Working Group: Lung Environment Interaction

Abstract No. 113

Emphysema Detection with Proton PREFUL MRI Parametric Response Mapping (PRM)

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PURPOSE: This study aimed to establish an MRI method analogous to CT parametric response mapping (PRM) for classifying lung ventilation abnormalities, such as emphysema and air-trapping, and to compare it with CT-PRM.
METHODS: A retrospective analysis of 34 subjects from the COSYCONET study was performed. PREFUL-PRM was calculated for four coronal slices using a spoiled gradient echo sequence during free breathing. Post-processing involved several steps prior to pseudo HU scaling and PRM, including field inhomogeneity and signal decay correction. CT was aquired in full in- and expiration. Slices were matched and averaged to PREFUL slice thickness prior to inter-modality registration. Regional and total lung percentages results were assessed.
RESULTS: Visual agreement and regional correspondence were confirmed between both PRM methods (overlap normal: 91(83 - 99)%; fSAD 47(29 - 58)%; emphysema 17(3 - 29)%). High correlations were found for total-lung-values across all three classes (r>=0.86), but with significantly different median values (P<0.001) in a similar range. The mean biases were: CT/PREFUL Normal: 73.50%/80.77%, bias -7.58; fSAD: 24.11%/18.31%, bias 6.39; Emphysema: 1.74%/0.23%, bias 1.19.
DISCUSSION : The novel method allows for similar detection of fSAD and emphysema using PREFUL MRI as with CT. The low overlap for emphysema can be explained by low amount of such regions. Furthermore, differences between MRI and CT can be caused by different respiration maneuvers (free-breathing vs. breath-hold). Improvements can be made in acquisition, bias field correction, and HU mapping, potentially utilizing deep-learning.
CONCLUSION: PREFUL-PRM can classify three ventilation states (normal, fSAD, emphysema) with high agreement to CT, potentially adding value to functional lung MRI.

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Abstract No. 114

Adeno associated virus (AAV) and CRISPR-Cas9 targeted inhibition of cholesterol metabolizing enzyme CH25H to reduce iBALT formation and prevent COPD progression

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This abstract is under embargo due to IP-relevant content

Contribution of a Disease-spanning Working Group: Lung Environment Interaction

Abstract No. 115

Pro-coagulant extracellular vesicles mediate smoking-induced pulmo-vascular inflammation

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Background: Chronic obstructive pulmonary disease (COPD) is a leading cause of death worldwide, with smoking as major risk factor. Tobacco smoke increases the risk of thrombosis, resulting in dysregulation of tissue factor (TF)-mediated extrinsic coagulation that further impacts downstream intracellular signaling via protease activated receptors (PARs). Here, we investigated the impact of bronchial epithelial cell (BEC)-derived TF^+-extracellular vesicles (EVs) on human lung endothelial cell (EC) activation as mediators of pulmo-vascular inflammation.
Results: We could show that human BECs secrete pro-coagulant TF^+-EVs when stimulated with tobacco smoke extract. Those TF^+-EVs are secreted towards the lung lumen and basolateral side by polarized BECs to potentially target PAR1 and PAR2 expressing cells from the lung lumen and tissue side. COPD-specific regulation of TF and PAR1 was further demonstrated by re-analysis of public RNA sequencing data showing upregulation of these factors in lung tissue from COPD patients. With respect to potential TF^+-EV target cells, we demonstrated that especially lung ECs express PAR1 and PAR2. Stimulation of these cells with TF^+-EVs induced EC activation as monitored by proinflammatory gene expression of IL-8, ICAM-1 and VCAM-1 or intracellular calcium release. Additionally, EC activation can be blocked by PAR1 and PAR1/PAR2 siRNA knockdown as well as the clinically relevant thrombin-inhibiting anti-coagulants Dabigatran and anti-thrombin III.
Conclusion: In conclusion, tobacco smoke induces secretion of procoagulant TF^+-EVs by BECs to stimulate human lung ECs via PAR1 and PAR2. Stimulated lung ECs express pro-inflammatory mediators, thereby potentially promoting COPD-associated EC dysfunction and thrombotic events.

Abstract No. 116

Combining endobronchial and intrabronchial valves for the endoscopic therapy of advanced emphysema

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Background: Endoscopic valve implantation is a treatment option for selected patients with advanced emphysema. For this purpose, endobronchial (EBV) or intrabronchial valves (IBV) can be used. We have analysed the results of treatment after combining the two available valve types.
Methods: Retrospective analysis of patients who received combined therapy using IBV (Spiration-Olympus, Tokyo, Japan) and EBV (Pulmonx, Neuchâtel, Switzerland) at the Thoraxklinik Heidelberg from 02/13 to 11/21. The decision regarding the selection and combination of valves was made by the investigators during bronchoscopy, based on the special anatomy of the bronchi. The effectiveness and complications were analysed at 90d-FU.
Results: 89 patients (♂/♀:55%/45%, age: 65±7 years) were included. In 95.4±7.2% of the cases the interlobar fissures were visually intact. During the intervention, a mean of 1.8±0.85 EBV and 1.2±0.42 IBV pro patient were implanted. IBV were predominantly implanted in the lower lobes, 88% of them in segment six. FU showed target lobar atelectasis in 53% of cases. In the lung function testing, there was significant improvement in FEV_1 (0.75±0.22L vs 0.84±0.27L, $p<0.00001$), RV (5.7±1.45L vs 5.0±1.41L, $p<0.00001$), 6MWD (266±109m vs 302±91m, $p<0.0001$) and mMRC (3.2±0.9 vs 2.7±1.3, $p=0.0021$). Pneumothoraces occurred in 11 cases, 6 requiring drainage. A total of 23 pneumonias occurred, 11 with hospitalization. 4 patients reported self-limiting haemoptysis.
Conclusion: Combined implantation of IBVs and EBVs resulted in atelectasis of the target lobe in the majority of cases with statistically significant clinical and functional improvement with an acceptable risk profile. The combination of both valves expands the possibilities of valve therapy according to bronchus anatomy.

Abstract No. 117

Induction of telomerase in p21-positive cells counteracts capillaries rarefaction and protects against lung emphysema in mice.

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Variations in telomere state are critical for cellular senescence and the development of lung diseases that increase in frequency with age, particularly pulmonary emphysema and pulmonary fibrosis
We have generated a mouse model in which telomerase (mTert), or its catalytically inactive form (mTertCI), is expressed from the p21Cdkn1a promoter. We found that this particular expression of Tert curbs age-related emphysema and pulmonary perivascular fibrosis in aged mice and reduces age-dependent accumulation of senescent cells, many of which are endothelial cells (EC). We further show that Tert counteracts age-related decline in capillary density and promotes maintenance of high numbers of Cd34+ cells identified as a subclass of EC with proliferative capacity. In addition, young p21+/mTert mice exposed to hypoxia and treated with the VEGF receptor inhibitor SUGEN were protected against loss of capillary density and the development of pulmonary emphysema. Importantly, expression of mTertCI did not prevent EC senescence, decreased capillary density, and development of emphysema, although mTert and mTertCI significantly reduced p21 levels in old mouse lungs. Taken together, these results.
Collectively, these results show that mTert reduces age-related lung function decline by promoting regeneration of the microvasculature and by preventing replicative senescence.

Abstract No. 118

FGF10 treatment activates repair mechanisms of lung alveolar and vascular structures in mice after established emphysema and PH

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Chronic obstructive pulmonary disease (COPD) is a serious medical condition, comprising emphysema, chronic bronchitis and often pulmonary hypertension (PH). We recently identified impaired fibroblast growth factor (FGF) 10 signalling as an integral part of cigarette smoke (CS)-induced emphysema and PH development. Mice with impaired FGF10 signalling spontaneously developed emphysema and PH, associated with vascular pruning and remodelling. Moreover, FGF10 treatment reversed established CS-induced emphysema and PH in mice. However, the exact mechanism of FGF10-mediated repair of lung alveolar and vascular structures remains elusive.

In lungs of experimental mice, we investigated alveolar epithelial type 2 (AT2) cells and endothelial cells (ECs) that express FGF10 receptor. FGF10 treatment, after established CS-induced emphysema and PH, led to an increase in AT2 cell number and an increase/expansion of EC, reversing the disease. Moreover, we found β-catenin as a potential molecular mechanism downstream of FGF10 treatment. These results from experimental animals were verified in human COPD precision cut lung slices, treated *in vitro* with recombinant FGF10. Furthermore, using cell-specific knockout mice we aim to investigate the role of FGF10 in lung homeostasis and repair.

In addition, transcriptomic analysis of mouse lungs revealed TOX High Mobility Group Box Family Member 2 (TOX2) as a novel potential driver of FGF10-mediated reverse remodelling in the pulmonary vasculature. Available single cell sequencing data indeed pinpoints TOX2 expression in pulmonary arterial ECs. This was further verified in lung sections of experimental animals and using *in vitro* EC models. However, more research is needed to decipher the role of TOX2 in vascular remodelling/reversion.

Contribution of a Disease-spanning Working Group: Lung Environment Interaction

Abstract No. 119

Immune cell profiling in combination with machine learning approaches defines two clinically distinct COPD subtypes

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Rationale: COPD presents with multiple overlapping clinical phenotypes and underlying endotypes. The immune system plays a multifactorial role in disease pathogenesis and progression. However, it is unclear how the local immune-environment associates with the disease's heterogeneous manifestations. Therefore, we sought to quantify and stratify the local immune cell environment in end-stage COPD lungs in comparison to control donor lungs.

Methods: Computational flow cytometry and cytokine multiplex assays were performed on lung explants of COPD patients and donors/controls and analysed bioinformatically. Machine learning algorithms were applied to identify potential subtypes.

Results: COPD lungs possessed a distinct immune cell profile as compared to donors defined by random forest (RF) models (AUC=0.93). Increased lymphocytic populations in COPD lungs and abundant monocytes and PMN-L in donor lungs drove the RF model. Closer examination of the COPD cohort revealed two subtypes based on their specific lung immune profile. The first patient subtype was characterised by elevated $CD8^+$ T cells, dendritic cells and circulating IL1β, TNFα, and IFNβ levels. Clinically they were slightly younger, had more pronounced airspace enlargement, exhibited lower oxygen saturation, and thus termed "adaptive-immune severe". In contrast, the patients in the second subtype, termed "innate-immune mild", were older, possessed milder airspace enlargement, higher oxygen saturation, and showed enrichment of monocytes, macrophages, and CCL11, CXCL1, CCL17 plasma levels.

Conclusions: Our multilevel immunophenotyping approach identified two COPD subtypes and their associated circulating cytokine profiles. This may provide future options to stratify COPD patients and targeted personalized treatment.

Abstract No. 120

Initial events within the COPD pathogenesis triggered by whole cigarette smoke exposure in primary human bronchial epithelial cells

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Chronic obstructive pulmonary disease (COPD) is a highly prevalent progressive lung disease characterised by emphysema, chronic inflammation, airway remodelling, and mucus hypersecretion. Given the limited success of current treatments, open research aims to gain insights into early disease mechanisms ([1]). We hypothesize that cigarette smoke (CS) exposure induces a dysregulation of airway basal stem cells (BSCs) in pHBECs contributing to the pathogenesis of COPD ([2]). To clarify disease mechanisms, we exposed primary human bronchial epithelial cells (pHBECs) of three different donors for 28 days to CS at an air-liquid-interface using the ExpoCube ® and took samples for viability, transepithelial electrical resistance (TEER) measurements, cellular differentiation, cytokine secretion, and gene expression levels on day 7, 14, 21, and 28. Chronically CS-exposed pHBEC cultures indicated a lower TEER on day 21 (control (455Ω) vs CS (233Ω)) and day 28 (control (492Ω) vs CS (354Ω)) with a reduction of 49% and 30%, respectively. This points towards changes in the differentiation status. In accordance with that, we also observed changes in the secretion of epidermal growth factors Epiregulin and Amphiregulin which were elevated from day 7-28. The expression of squamous metaplasia gene markers IVL (1,68x) and KRT13 (3,45x) was increased on day 28. Further analysis of the CS-challenged BSCs transcriptome will clarify the biological mechanisms driving the deterioration of BSC biology. These results give first insights into the chronological regulation of BSC-specific markers after chronic CS exposure of primary human bronchial epithelial cells.

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Abstract No. 121

Influence of metronome-paced tachypnea on ventilation in COPD patients and healthy subjects

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Introduction:
Metronome-paced tachypnea (MPT) induces dynamic hyperinflation (DH) in patients with chronic obstructive pulmonary disease (COPD). Purpose of this study was to test the effect of MPT on lung ventilation for repeatability and to compare between healthy subjects and COPD patients using phase-resolved functional lung (PREFUL)-MRI. [1]
Methods:
24 patients with moderate to severe COPD as well as 30 healthy subjects were examined in a single center study. A 90s long time series of one coronal slice centered to the trachea was acquired for PREFUL-MRI during 60s of rested tidal breathing (RTB) and 30s of MPT at 40 breaths / min. To assess repeatability, the maneuver was performed twice. Two ventilation parameters, Fractional Ventilation (FV) and End-Expiratory Lung Area (EELA), were calculated. The ratios of FV (MPT/RTB) and EELA (end/start of MPT) were formed and compared between healthy subjects and COPD patients.
Results:
FV decreases significantly during MPT in both healthy subjects ($p=0.011$) and COPD patients ($p<0.001$). However, the effect of MPT is significantly stronger in COPD patients ($p=0.01$). EELA is not affected by MPT in healthy subjects ($p=0.153$) but rises significantly in COPD patients ($p=0.004$). In healthy subjects both parameters show moderate to high repeatability. The repeatability is worse in COPD patients.
Discussion:
A significantly stronger effect of MPT on ventilation was observed in COPD patients when compared to healthy subjects. Ventilation measurements during MPT are sufficiently repeatable in healthy subjects and COPD patients. However, sufficient recovery time is necessary for COPD patients to return to normal levels of hyperinflation. [2]

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Abstract No. 122

Are murine air liquid interface (ALI) models suitable for toxicity testing of cigarette smoke extract (CSE)?

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Objective: Cigarette smoke exposure is one of the main causes of Chronic Obstructive Pulmonary Disease (COPD). Application of Cigarette Smoke Extract (CSE) during the differentiation of Human Bronchial Epithelial Cells (HBEC) at the Air Liquid Interface (ALI) decreases the number of ciliated cells, while the populations of club and goblet cells are increased [1]. ALI models from available gene-deficient mice will be helpful for a detailed molecular and cellular analysis of these pathways. Therefore, we asked, if primary murine tracheal epithelial cells (mTec) cells differentiated in a murine ALI model behave similar to the human model after exposure to CSE.
Methods: MTec containing basal cells from wild-type mice were isolated, cultured and differentiated to a pseudostratified epithelium in an ALI model. CSE (5%) was added to the medium in the basal chamber and mRNA expression as well as cells were analyzed as described [2].
Results and Outlook: We established a reproducible protocol for ALI-cultures of murine tracheal basal cells. MRNA expression of smoke exposure regulated genes (SERGs) was up-regulated by CSE in a murine similar to the human ALI model. Cell viability assays show no acute toxicity. Identification of epithelial cells was performed by IF-staining, and revealed no altered cell differentiation pattern after chronic CSE application. However, at the mRNA level, we found a downregulation of cell specific markers. Our data in the murine ALI model show no differences in cell numbers after application of CSE compared to the human model. Therefore, murine ALI models may be not suitable to dissect molecular steps in CSE-associated diseases like COPD.

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Abstract No. 123

Spatially-resolved LCM-MS niche proteomics uncovers a potential role of microvasculopathy in COPD progression

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Chronic obstructive pulmonary disease (COPD) is currently the third leading cause of death. It is characterized by emphysema and chronic bronchitis, which leads to extreme degeneration of alveolar structures and breathing problems at the end stage. Key events during COPD progression, such as the loss of small airways has been reported by many CT-imaging studies. However, the dynamic changes of cellular circuits and proteomic composition of microanatomical niches during disease progression remains poorly characterized.
Here, we profiled non-smoker control (n=4), smoker control (n=3), COPD GOLD stage-II (n=4) and COPD GOLD stage-IV (n=5) patients with both spatially-resolved laser-capture microdissection-coupled mass spectrometry (LCM-MS) and single-nucleus transcriptomics (snRNA-Seq). The integrated analysis of both data modalities in matched donor samples is used to characterize tissue niche changes in COPD disease progression. For spatial LCM-MS proteomics, we characterized the small airway (terminal and respiratory bronchioles), the alveolar septum and distal vasculature (microvessels, pulmonary arteries and veins) and traced histopathological changes of these structures during progression. In total, we profiled 135 samples covering 38 regions of interest via mass spectrometry, which yielded 6770 proteins (including 418 matrisome proteins). In addition, 48301 nuclei were sequenced from matching donors.
Combining the power of spatial proteomics and snRNA-Seq transcriptomics, we aim to identify spatial niches that differ across COPD stages. We observe proteomic changes in the lung parenchyma and vasculature during the disease progression and verify if these trends are reflected in the snRNA cells. These findings will be validated by advanced imaging methods.

Contribution of a Disease-spanning Working Group: Lung Environment Interaction

Abstract No. 124

Reproducibility and outcomes of comorbidity clusters in patients with COPD

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Most patients with chronic obstructive pulmonary disease (COPD) exhibit comorbid conditions. While individual comorbidities contribute to disease severity and poor prognosis, the association of comorbidity patterns with severity and outcomes of COPD is unclear. Previously, five clusters with distinct comorbidity profiles were identified among patients from the CIROCO COPD cohort. To validate the comorbidity clusters in a larger, independent cohort (COSYCONET) and to investigate their association with disease severity and prognostic outcomes.
Data from CIROCO and COSYCONET were integrated. Comorbidity information of COSYCONET GOLD 1-4 patients (n = 1,052) was used to generate a new cluster model using machine learning. Reproducibility of comorbidity clusters, as well as their association with disease severity and prognostic outcomes were investigated.
Four out of five comorbidity clusters (less comorbidity, cardiovascular, cachectic and metabolic) were reproduced, while a psychologic cluster could not be reliably assessed due to limited data availability. Cross-sectionally, clusters differed significantly in airflow obstruction, hyperinflation and diffusion capacity, with most severe impairment in the cachectic cluster. While no cluster specific differences in mortality were observed after 1.5 and 4.5 years of follow-up, patients from the less comorbidities cluster had the most pronounced lung function decline. Furthermore, patients from the cachectic cluster had an increased frequency of severe acute exacerbations. Comorbidity clusters are reproducible between independent COPD cohorts recruited in primary and tertiary care settings. The less comorbidity and cachectic clusters were associated with lung function decline and increased exacerbations, respectively.

Abstract No. 125

Perinatal obesity induces premature alveolar aging by ATII cell depletion via IL-6-driven transcriptional and genome instability

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Background: Obesity is closely associated with cardiopulmonary diseases. Despite Obesity and aging converging in similar pathways, specifically inflammation-associated genomic instability, their interdependency remains unclear. We recently demonstrated that perinatal obesity triggers bronchial and vascular remodeling through Interleukin 6 (IL-6).
Aims: We investigated if and how perinatal obesity induces early genomic instability in alveolar epithelial cells type 2 (ATII) leading to alveolar simplification
Methods: Lungs of offspring of high-fat diet (HFD)- or standard-diet (SD)-fed dams were analyzed at postnatal day 21 (P21), P70, P365 and after 1.5 years, including global and ATII-specific transcriptomic and epigenetic profiling. Cultured ATII, precision-cut lung slices (PCLS) and bronchioalveolar lung organoids (BALOs) were used for *ex vivo* studies.
Results: Perinatal obesity induced a life-long emphysema-like lung structure with a reduction of ATII, increased DDR ($\gamma H2AX^+$) and senescence ($p21^+$). Global and ATII-specific RNA-Seq together with ATII-specific ATAC-Seq identified DNA damage and repair processes together with transcriptional stress and aging-associated pathways in ATII after perinatal obesity. Employment of cultured ATII, PCLS and BALOs demonstrated that WAT secretome inhibits growth and induces DDR, senescence and loss of ATII through IL-6. IL-6 null mice and mice with pharmacological inhibition of IL-6 signaling were protected from DDR and alveolar growth arrest after perinatal obesity. Finally, human lungs with emphysema showed increased DDR and loss of ATII.
Conclusion: Our data demonstrate that perinatal obesity induces genomic instability in AT2 with life-long emphysema-like lung structure, a process that is in part mediated by WAT derived IL-6.

Abstract No. 126

Exploring the pre-developmental phase of pulmonary hypertension in COPD: Insights into initial pulmonary vascular remodeling

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Pulmonary hypertension (PH) frequently develops in patients with chronic obstructive pulmonary disease (COPD). PH in COPD is characterized by increased pulmonary arterial pressure and remodeling of the pulmonary vasculature. Importantly, even mild PH can affect survival of the patients. Interestingly, pulmonary vascular alterations have been observed in smokers who had not developed COPD and therefore have been suggested to contribute to the development of emphysema. Nevertheless, the mechanisms involved are not yet fully understood. Previously, we demonstrated that chronic exposure to cigarette smoke (CS) in a mouse model leads to the development of pulmonary hypertension after three months, while emphysema is detectable only several months later. However, especially the early timepoints of disease development have not been investigated yet in detail.
Against this background, the present study aimed to observe the early alterations in the pulmonary vasculature that contribute to the development of PH in COPD. Accordingly, we exposed C57BL/6J mice to CS and room air (RA) for durations of 1 to 4 weeks. Our results indicated that 4 weeks of CS exposure induced the proliferation of pulmonary vascular cells, which might be the initial stage of vascular remodeling in the vessels. However, no differences were observed in the proliferation of pulmonary vascular cells after 1, 2, or 3 weeks of CS exposure when compared to the RA control.
Taken together, our observations indicate that the proliferation of pulmonary vascular cells may commence as early as 4 weeks after CS exposure. Our future aim is to study the specific transcriptomic signature of cells that drive the remodeling process of vessels using single-cell RNA sequencing.

Contribution of a Disease-spanning Working Group: Lung Environment Interaction

Abstract No. 127

Immunomodulation of age-dependent alveolar stem cell function

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Aging is the major risk factor for several chronic lung diseases such as Chronic Obstructive Pulmonary Disease (COPD). Hallmarks of aging, such as cellular senescence and inflammaging (an age-associated, chronic inflammatory state) contribute to a reduced regenerative potential of the lung. This is mediated by both cellular factors, e.g. by alveolar macrophages (AMs), as well as by various intrinsic and extrinsic soluble factors contributing to an anti-regenerative niche. To study these effects, we used a murine alveolar epithelial organoid model. First, we investigated the effect of alveolar macrophages (AMs) on stem cell function by generating immunocompetent organoids containing AMs. Immunofluorescence staining revealed the persistence of AMs throughout the culture period. The impact of aging was studied using AMs from young and old mice as well as ex vivo aged AMs, showing a decrease in colony forming efficiency (CFE) with age. Next, intrinsic soluble factors were studied by treating organoids with Bronchoalveolar Lavage Fluid (BALF) of old mice, which showed a significant decrease in CFE compared to young BALF. Treatment with cytokines (IL-6, IL-1β, TGFβ1, TNF-a) contained in the Senescence Associated Secretory Phenotype (SASP) showed significantly increased CFE in the IL-1β treatment, while TGFβ1 reduced CFE and organoid size. Furthermore, examining extrinsic mediators in cigarette smoke extract (CSE) treatment as an environmental factor leading to COPD, we observed a significant reduction in CFE and organoid size in 10% CSE treatment. Overall, we show an anti-regenerative effect of cellular and soluble inflammaging factors on alveolar stem cells and will employ the established models to further investigate age-related changes contributing to disease pathology.

Abstract No. 128

Assessment of lobar and segmental bronchial instability with time-resolved low dose 4DCT of the whole chest and correlation with pulmonary function tests in COPD

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COPD can be associated with airway collapsibility due to chronic inflammation and subsequent cartilage malacia. While tracheal collapsibility can be investigated with bronchoscopy, dynamic changes in peripheral bronchi remain unknown. This study aims to quantify the collapsibility of peripheral airways objectively using time-resolved 4DCT, and to correlate changes with clinical data and PFT.

Low-dose 4DCT of the whole chest was performed in 46 freely breathing patients with clinically suspected airway instability. 30 had COPD GOLD III-IV. The respiratory cycle was divided into 5%-wide steps, resulting in 21 3D datasets per patient. The bronchial tree was segmented semi-automatically for all datasets. Changes in volume (pBV) of lobar, segmental, and first subsegmental bronchi were objectified with in-house software. Correlations between airway changes and clinical data (GOLD stage), QCT of additional inspiratory CT (emphysema index), and spirometry (FEV1%) were investigated.

A significant change in pBV was found in 23 patients. Patients with COPD showed significantly higher collapsibility of pBV than the control group (mean reduction of pBV: 50% vs 36%; $P<0.05$). In COPD, changes in pBV occurred earlier and faster during expiration. Changes in pBV correlated with EI% ($r=0.69$; $P<0.01$) and inversely with FEV1% ($r=-0.58$; $P<0.01$). Significant collapsibility of the trachea did not predict instability in peripheral airways ($P>0.5$).

4DCT of the whole chest can detect respiratory dynamics of central and peripheral airways using software quantification. The correlation with PFT indicates a relevant influence of peripheral airway dynamics on disease severity in COPD patients. Further, the collapsibility of central airways seems to be independent of peripheral airway dynamics.

Abstract No. 129

Advanced lung function targeting small airway dysfunction (SAD) improves differentiation of mild COPD and smokers at risk from healthy controls –CAPTO-COPD

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Background: SAD is an early feature of COPD commonly overseen in routine diagnostics such as forced spirometry. Advanced tidal-breathing lung function testing showed potential to overcome this impediment. We aimed to assess their diagnostic value in differentiation of early disease using a machine learning-based approach.
Methods: We included 211 patients with mild COPD (post $FEV_1/VC<70\%$ + $FEV_1 \geq 70\%$pred), (ex)-smokers at risk ($FEV_1/VC \geq 70\%$, ≥10py + CAT≥10 or long-acting bronchodilator) from CAPTO-COPD observational study and healthy controls. Conventional tests included spirometry, body plethysmography and transfer factor (TLCO). Oscillometry (OS) and SF_6-multiple breath washout (MBW) were additionally performed. We compared the accuracy of random forest class prediction including parameters of (1) spirometry only, (2) spirometry + body plethysmography + TLCO, (3) spirometry + OS + MBW and (4) all. We retained a test set for accuracy evaluation and used cross-validation for model tuning.
Results: Final analysis was performed in 90 COPD patients (mean age 65±9 years, mean FEV_1 77±12%pred), 62 smokers at risk (60±10, 89±13) and 59 controls (45±19, 98±12, all $p<0.001$). Model accuracy was 63% (κ=42%) when using (1) spirometry only. Considerable improvement was achieved adding (2) conventional parameters (80%, κ=70%), (3) advanced parameters (78%, κ=66%) and (4) combining all approaches (85%, κ=77%, all $p<0.01$).
Conclusion: Machine learning-based differentiation of patients with only mild COPD and (ex)-smokers at risk from healthy controls can be considerably improved when including advanced lung function testing also targeting SAD.

Abstract No. 130

Characterization of lung function in coronary heart disease (CHD) and chronic obstructive pulmonary disease (COPD) – going beyond spirometry

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Background: Concomitant CHD and COPD is frequent while being associated with considerable morbidity and mortality. However, simultaneous diagnoses are often missed as widely used spirometry may be insensitive to emphysema or small airway disease (SAD). We therefore aimed to evaluate patients at risk using advanced lung function testing.
Methods: 192 stable patients (61±16 years, 72% former/active smoker) were classified in 4 groups according to previously diagnosed CHD or COPD. Spirometry (FEV_1/FVC), body plethysmography (RV/TLC), oscillometry (frequency dependence of resistance, D5-20), carbon monoxide transfer factor (TLCO) and echocardiography (EF, TAPSE) were retrospectively analysed.
Results: 58 patients had CHD, 21 COPD, 18 both diseases and 95 no history of CHD or COPD. RV/TLC was significantly increased in CHD in absence of known COPD indicating hyperinflation. D5-20 further increased in concomitant COPD and CHD as compared to COPD alone indicating SAD. TLCO reduction was mainly associated with COPD (ANOVA, $p<0.05$). EF was significantly reduced in patients with CHD whereas TAPSE was mainly reduced in COPD.

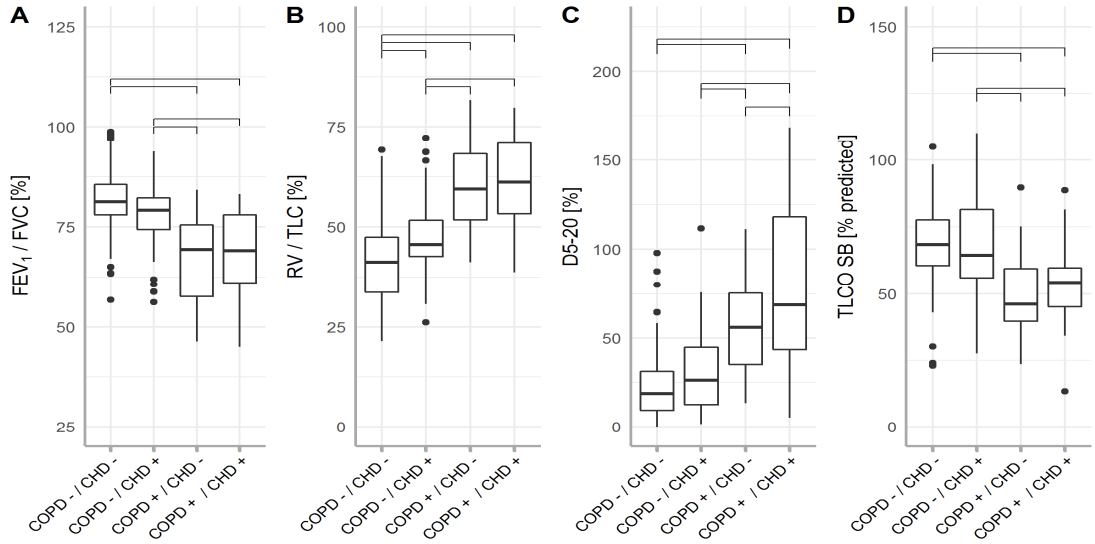

Figure 1

Conclusions: Our results suggest that features of airway disease can be revealed in patients at risk for concomitant CHD and COPD using advanced lung function testing. Prospective evaluation of hyperinflation and SAD seems promising and may outperform spirometry.

Abstract No. 131

Antiviral CD8+ T cell immune responses are impaired by cigarette smoke and in COPD

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Virus infections drive COPD exacerbations and contribute to COPD progression. Cigarette smoke dampens anti-viral immunity, which possibly contributes to defective responses to viral infections in COPD patients. Anti-viral immunity centers on the activation of virus-specific CD8+ T cells by viral epitopes presented on major histocompatibility complex (MHC) class I molecules of infected cells. These epitopes are generated by the immunoproteasome, a specialized type of proteasome, that is upregulated by interferons upon infection. The effect of cigarette smoke on induction of the immunoproteasome and MHC class I-mediated CD8+ T cell activation is unresolved.

We here demonstrate that cigarette smoke attenuates cytokine and virus-induced upregulation of the immunoproteasome in vitro, ex vivo and in vivo. Using a CD8+ T cell activation assay, we show diminished MHC class I-mediated activation of influenza A virus (IAV)-specific CD8+ T cells by cigarette smoke extract. Moreover, cigarette smoke profoundly altered the repertoire of inflammatory MHC class I antigens in lung epithelial cells as revealed by advanced mass-spectrometry-based immunopeptidome analysis. Of note, COPD patients have reduced numbers of circulating IAV-specific CD8+ T cells compared to healthy controls and asthmatics.

In conclusion, cigarette smoke causes defective immunoproteasome activation and MHC I antigen presentation in lung cells that results in impaired activation of CD8+ T cells upon virus infection. This may contribute to reduced viral clearance and increased susceptibility to viral exacerbations in COPD.

Abstract No. 132

Epithelial Lipocalin-2 synthesis after smoking and viral mimik?

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Although smoking is a major COPD risk factor, not all smokers develop the disease. Viral infections are more frequent and severe in smokers, potentially increasing the susceptibility to COPD. In a mouse model mimicking heavy smoking with viral infection, lipocalin-2 was upregulated in BALF. The upregulation was confirmed in murine lung epithelium as well as in human BALF cells and lung tissue from COPD patients. We aim to establish an in vitro model to investigate if the airway epithelium augments lipocalin-2 secretion upon smoking and viral infection.
Normal human bronchial epithelial (NHBE) cells from female donors were differentiated at the air-liquid interface and exposed to cigarette smoke (CS) or room air daily for 14 days (ExpoCube (SCIREQ, Montreal, Canada)). Control cells were not exposed. The basal and apical medium was collected every other day, and TEER measurements were performed. After the last CS exposure, cells were collected for protein, RNA, and histologic analyses. Cell viability was assessed by water soluble tetrazolium (WST) and lactate dehydrogenase (LDH) assays.
No major difference in cell viability was found between the groups. A significant decrease in the epithelial barrier integrity was detected in CS-exposed cells (1 cig. $p = 0.0002$, 2 cig. $p = 0.0007$, 3 cig. $p < 0.0001$; compared to air). Moreover, epithelial hyperplasia was observed in the cells following the CS-exposure.
NHBE cells remained viable regardless of the CS but had an impaired epithelial barrier integrity and structural damage to the cell layer. The structure of the epithelial cell layer was increasingly altered with increasing CS dose. Testing varying cigarette numbers established in vitro smoking model. Next, the cells will be infected with hRV16 to analyze lipocalin-2 secretion.

Contribution of a Disease-spanning Working Group: Lung Environment Interaction

Abstract No. 133

CEACAM6: A genetic determinant and therapeutic target for alleviating nitrosative/oxidative stress in COPD patients

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Chronic obstructive pulmonary disease (COPD), a global health problem, stems primarily from exposure to noxious substances in cigarette smoke (CS) and polluted air. One of main pathomechanisms is nitrosative/oxidative stress. However, it remains poorly investigated which mechanisms underlie the defence against nitrosative/oxidative stress in the majority of smokers who do not develop COPD.
Against these backgrounds, we established an *in vitro* model of CS extract (CSE)-resistant alveolar epithelial cell line, which showed decreased nitrosative/oxidative stress, but increased levels of antioxidant protein – heme oxygenase-1 (HO-1). Moreover, transcriptomic profiles between control and R revealed carcinoembryonic antigen cell adhesion molecule 6 (CEACAM6) as the repressor of HO-1. The results were validated as CEACAM6 protein levels inversely correlated with HO-1 in human alveolar type 2 epithelial cells (hAEC2). To consolidate the role of CEACAM6 in COPD, we performed *ex vivo* experiments using human healthy donor precision-cut lung slices (hPCLS). The results demonstrated that epithelium-specific CEACAM6 overexpression increased nitrosative/oxidative stress and cell death upon CSE treatment. Strikingly, CEACAM6 protein levels in human peripheral blood mononuclear cells inversely correlated with disease severity, suggesting that CEACAM6 is a genetic factor and a potential biomarker in COPD.
Taken together, we provided evidence that CEACAM6 is a new regulator of HO-1, hampering antioxidant defence directly in lung epithelial cells. CEACAM6 may present as a susceptibility determinant and a potential biomarker in COPD. Targeting CEACAM6/HO-1 axis can be a novel therapeutic approach to alleviate nitrosative/oxidative stress in patients with COPD.

Abstract No. 134

Levels of autoantibodies against human bronchial epithelial cells are elevated in patients with COPD and associated with levels of circulating chemokines

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A growing body of evidence supports for an association between chronic obstructive pulmonary disease (COPD) and autoimmunity. However, it remains unclear whether the autoimmunity contributes to the development of COPD, and if yes, what is the pathomechanism underlying its contribution. Here we hypothesize that functional autoantibodies against cell surface antigens act directly on lung cells and contribute to the development of COPD. To validate this hypothesis, we determined autoantibodies of IgM and IgG classes against primary human bronchial epithelial cells (HBEC) and lung fibroblast in a 147 COPD patients and 64 age-matched healthy controls. Our results showed that both IgM and IgG autoantibodies against primary HBEC were significantly increased in patients with COPD compared to healthy subjects. By contrast, negligible levels of IgM and IgG autoantibodies against primary human lung fibroblast were detected in both COPD patients and controls. Moreover, levels of IgM autoantibodies against primary HBEC in patients with COPD were correlated with levels of circulating CCL3, CCL4, CCL20 and CXCL9. Taken together, our finding demonstrates an association of autoantibodies against HBEC with COPD and circulating chemokines.

Abstract No. 135

Ambient ozone is related to faster lung function decline: The ECRHS study

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Background:
Short-term exposure to ambient ozone shows adverse effects on lung function. However, whether long-term exposure also has detrimental effects on lung function is still unclear.
Objective:
We explored the association between long-term exposure to ozone and lung function over 20 years

in 3014 adults from 17 centers in 8 countries participating in European Community Respiratory Health Survey (ECRHS).

Methods:

Pre-bronchodilation FEV1 and FVC were measured by spirometry when participants were around 35, 45, and 55 years old. Annual mean values of daily maximum running 8-hour average ozone concentrations were assigned to the residential addresses. Other pollutants ($PM_{2.5}$ and NO_2) and greenness (Normalized Difference Vegetation Index, NDVI) were also considered for adjustment. Associations between lung function and ozone were analyzed by adjusted linear mixed effects regression models. An interaction term between the ozone and age was used to capture the change of the lung function.

Results:

Mean ozone concentrations were approximately 65 µg/m³. An increase an interquartile range of 7 µg/m³ concentration of ozone was associated with a faster decline in FEV1 of -2.08 mL/year (95% confidence interval: -2.79, -1.36) and in FVC of -2.86 mL/year (-3.73, -1.99) mL/year. Associations were robust across different models, including adjustments for co-pollutants and greenness, and more pronounced in residents of northern Europe and those with higher education or older than 35 years. No consistent associations were detected with the FEV1/FVC ratio were found.

Conclusion:

Long-term exposure to elevated ambient ozone concentrations was associated with a faster decline of spirometric lung function over 20 years in middle-aged European adults.

Abstract No. 136

Cigarette smoke induces transient and persistent lung proteome changes

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Rationale: The airway epithelium is the first defence against cigarette smoke (CS), a major risk factor for chronic lung disease (CLD). Studying CS-induced protein changes may identify new therapeutic and preventive strategies for CLD, but few proteomic studies on patient-derived samples exist.
Objective: To identify CS-induced effects on the proteome of normal primary human bronchial epithelial cells (phBECs)[1] and in bronchoalveolar lavage fluid (BALF) of current, former, and never smokers to ultimately derive novel molecular pathways with relevance for CLD.
Methods: PhBECs were exposed to CS extract (CSE) followed by MS/MS-based proteomics. BALF proteomics data[2] was compared with respect to smoking history. Cytotoxicity (LDH) and barrier integrity (TEER) were monitored after ZZW115-mediated inhibition of Nuclear protein 1 (NUPR1) in phBECs (+/- CSE). Gene expression was analysed by qPCR and cellular localization by immunofluorescent stainings.
Results: CS induced transient and persistent changes in BALF with an overlap between transient changes in CS-exposed phBECs and BALF. Macrophages were a source of persistently altered proteins. Inhibition of NUPR1 induced cytotoxicity and pro-ferroptotic gene expression similarly in control and CS-exposed cells.
Conclusions: CS causes transient and persistent protein changes in BALF. Our findings suggest bronchial epithelial cells as source of the transient and macrophages as source of the persistent changes. CS does not significantly alter NUPR1 function in normal phBECs. Discovering disease- and cell type-specific mechanisms underlying the persistent response to CS may lead to the development of new CLD treatment approaches.

References:
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DA Cystic Fibrosis and Bronchiectasis: Abstract No. 201 – 230

Abstract No. 201

Clinical Feasibility of Multiple Breath Washout (MBW) in Patients with Cystic Fibrosis

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Background: Pulmonary function tests play an important role in diagnosis, management and treatment of patients with cystic fibrosis (CF). Multiple-breath washout (MBW) is a sensitive method to detect ventilation inhomogeneities and is established as a clinical tool in children with CF. Studies indicate promising data in adults with pulmonary diseases, but feasibility in adults is still questioned. We aim to analyse clinical feasibility of MBW in patients with CF and healthy controls (HC) and further evaluate factors influencing test duration.
Methods: We analysed a total of 1395 MBW measurements from CF patients (n=1158) and HC (n=237). CF patients were subdivided into high FEV1 (FEV1>90%), mid FEV1 (FEV1 40-90%) and low FEV1 (FEV1<40%) and age groups. Feasibility was defined as two acceptable trials in under 20 min. Influencing constitutional and disease severity factors were analysed by Spearman correlation.
Results: Clinical feasibility was 100% in HC and 92% in patients with CF, with higher feasibility within 20 min in children (96.0%) than adults (87.7%, p=0.01) with CF. In CF, Clinical feasibility decreased with decreasing FEV1 (96.6% in high, 92.8% in mid, 58.7% in low FEV1, p<0.05). The higher the FEV1, the larger is the influence of constitutional parameters (r=0.45 in low to 0.61 in high FEV1) and the smaller is the impact of severity factors (r=0.56 in low to 0.39 in high FEV1).
Conclusion: This study demonstrates that MBW is feasible in children and adults with CF. Feasibility is lower in patients with severe lung disease, but still 58% of these patients successfully complete a MBW test. Constitution has a major influence on test time in patients with mild disease. In contrast, disease severity factors outweigh constitution in influence, in severe disease.

Abstract No. 202

Culture medium-dependent effects of ivacaftor on the rescue of F508del-CFTR in primary nasal epithelial cultures from patients with cystic fibrosis

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Background
The introduction of highly effective CFTR modulator therapy with elexacaftor/tezacaftor/ivacaftor (ETI) led to a substantial clinical benefit in patients with cystic fibrosis (CF). In vitro studies suggest that potentiator compound ivacaftor as part of ETI triple combination therapy may impair correction efficiency of F508del-CFTR, however, quantitative studies in primary human airway epithelial cultures remain limited.
Methods
Primary nasal epithelial cells from 13 patients homozygous for F508del-CFTR were cultured using two common differentiation media: UNC or PneumaCult medium. Differentiated cultures were pre-treated with (i) elexacaftor/tezacaftor (ET); (ii) ETI; or (iii) DMSO (vehicle control). Rescue of CFTR chloride channel function was assessed by transepithelial short-circuit current (Isc) measurements and effects on mucociliary transport rates (MCT) were assessed by videomicroscopy.
Results
CFTR-mediated currents were significantly increased in both culture media by ET or ETI treatment compared to DMSO. In UNC medium, chronic presence of ivacaftor had no effect on amiloride-insensitive Isc, but reduced forskolin-induced and CFTRinh172-sensitive Isc. In PneumaCult medium, chronic presence of ivacaftor increased amiloride-insensitive Isc and decreased forskolin-induced Isc with similar CFTRinh172-sensitive Isc compared to ET. Furthermore, ETI-treated PneumaCult cultures displayed enhanced MCT compared to ET and DMSO.
Conclusion
These data show that culture medium composition affects functional F508del-CFTR rescue in vitro. Increased amiloride-insensitive Isc and increased MCT in ETI- vs. ET-treated PneumaCult cultures suggest that the presence of ivacaftor is important for constitutive CFTR activation and improvement of mucociliary clearance.

Abstract No. 203

Multiple-breath washout for the detection of lung disease in patients with inborn errors of immunity

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Introduction: Pulmonary manifestations are the major cause of morbidity and mortality in patients with inborn errors of immunity (IEI). Early recognition and treatment can improve outcome but requires sensitive diagnostic methods. Multiple-breath washout (MBW) is already established for the assessment of early lung disease prior to clinical signs and symptoms in patients with cystic fibrosis (CF).
Aims and objectives: The aim of this study was to compare MBW and spirometry in patients with IEI and CF as well as healthy controls (HC) and to evaluate the sensitivity of lung clearance index (LCI) to detect IEI lung disease.
Methods: Patients were recruited from our pediatric and adult immunodeficiency outpatient clinics. In addition, control groups of CF patients and HC were included. Nitrogen-MBW measurement and spirometry were performed with the study participants.
Results: In total, 342 participants (114 IEI, 114 CF, 114 HC) with a median (range) age of 21.0 (3.7-76.9) years were included. LCI ($p<0.001$) and forced expiratory volume in 1 second (FEV1) z-score ($p<0.001$) differed significantly between the groups and had a median (interquartile range (IQR)) of 7.0 (6.5-7.9) and -0.6 (-1.4-0.1) in the IEI group, 8.9 (6.8-14.2) and -1.1 (-2.7--0.3) in the CF group and 6.5 (6.2-6.9) and -0.1 (-0.8-0.5) in the HC group. Nineteen (20.7%) of 92 IEI patients and 35 (33.3%) of 105 CF patients had an increased LCI but a normal FEV1 z-score.
Conclusion: We demonstrated that LCI can detect lung disease in patients with IEI and appears to be more sensitive than FEV1. Future studies will investigate longitudinal measurements as well as the correlation with radiological imaging methods in different subgroups of IEI patients.

Abstract No. 204

Modelling chronic Pseudomonas aeruginosa lung infection with human airway cells at air-liquid interface

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The opportunistic human pathogen *Pseudomonas aeruginosa* causes chronic lung infections in susceptible individuals such as COPD and cystic fibrosis patients. *In vitro* models of *P. aeruginosa* infection are mostly confined to short time periods due to high bacterial cytotoxicity, limiting researchers' understanding of host-pathogen interactions during chronic infection.

To overcome this issue, we established a new model system of *P. aeruginosa* infection using both primary (normal bronchial epithelial) cells as well as a lung epithelial cell line (Calu-3), grown at the air-liquid interface and infected with well characterized, patient-adapted clinical isolates from the BACTOME database.

Bacterial load, cell viability, pro-inflammatory cytokine secretion and gene expression were analysed between 6 hours and 5 days after infection. Despite high bacterial loads, infected cells maintained up to 95% viability and showed elevated immune markers (including IL-1β, IL-6, IL-8 & G-CSF) compared to uninfected controls, up to 5 days post-infection. Concurrently, H&E staining confirmed an intact polarized monolayer throughout infection. Transcriptome analysis revealed upregulation of important *P. aeruginosa* virulence factors such as type II secretion system and iron transport systems.

We propose this human cell model to be used for future host-pathogen studies and compound screenings as a physiologically relevant addition to standard antimicrobial testing. The described method can be performed in a basic laboratory setting and allows monitoring of cellular and bacterial parameters.

Abstract No. 205

Airway commensal bacteria can inhibit the growth of cystic fibrosis pathogens via acetate production

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Around 90% of deaths in from cystic fibrosis disease (CF) result from pulmonary dysfunction that is directly linked with chronic infections. Although CFTR modulators have shown significant improvements in pulmonary exacerbations and lung function, their impacts on infection and disease progression are yet to be demonstrated. We thought to explore commensal-host-pathogen interactions. We had previously isolated commensal upper airway bacteria that interfere with growth of CF pathogens.
Various CF pathogens including *Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis, Achromobacter xylosoxidans, Haemophilus influenzae,* and *Stenotrophomonas maltophilia* were grown in conditioned media (CM) obtained from inhibitory commensal bacteria. Metabolomics profiling of the CMs from selected inhibitory vs. non-inhibitory commensal isolates was performed via high-performance liquid chromatography.
CMs from inhibitory commensal isolates inhibited the growth of the various CF pathogens tested. Inhibitory CMs had pH values close to 5.0, however a pH of 5.0 alone was not sufficient to inhibit pathogen growth. Neither heat treatment nor proteinase K treatment altered the observed CM inhibition. Metabolomics profiling revealed significant amounts of acetate production (> 0.5 mg/ml) in the CMs. Exogenous addition of 0.5 mg/ml acetate into the growth medium at pH 5.0 inhibited the pathogen growth.
This study demonstrates that airway commensals may be protective in the airway by affecting pathogenic growth via the production of short-chain fatty acids and suggest a potential use as postbiotic therapeutic approach whereby certain metabolites or nutrients can be administered to modulate the microbiome and thereby tip the host-commensal-pathogen balance towards health.

Abstract No. 206

Highly-effective CFTR-modulator therapy with elexacaftor/tezacaftor/ivacaftor (ETI) leads to age-dependent changes of CF microbiology - data from the German CF registry

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Background: The impressive improvements of CFTR function by elexacaftor/tezacaftor/ivacaftor (ETI) result in remarkable changes of pulmonary function and structural lung disease. Whether these effects translate to changes of microbial patterns remains to be shown. We thus assessed the effect of ETI on Staphylococcus aureus (SA) and Pseudomonas aeruginosa (PA) detections in people with CF (pwCF) >12 years.
Methods: We obtained data on SA and PA detections in throat swabs or sputa from the German CF Registry from January 2016 until July 2022 from people with CF (pwCF) with at least five microbiological analyses within three years before and at least one microbiological analysis in up to nine months after their initiation of ETI. We compared overall SA and PA detections and addressed individual changes of colonization status by descriptive statistics and logistic regression analyses.
Results: We included data from 2,319 pwCF with 26,349 microbiological samples prior to and 6,632 microbiological samples after initiation of ETI. Detections of SA decreased from 51.6% prior to ETI therapy to 41.5%, 42.5% and 42.8% and those of PA from 38.1% to 30.2%, 25.8% and 25.3% 3, 6 and 9 months after initiation of therapy, respectively (all $p<0.001$). Reduction of detections of SA and PA associated significantly with age and were dependent on pre-ETI colonization status. Pre-ETI colonization status did not impact upon ppFEV1 or BMI improvements.
Conclusions: The different patterns of reductions of SA and PA indicate that pathogen-specific biological processes govern the responsiveness of microbiological colonization towards ETI in pwCF.

Abstract No. 207

Characterization of the innate lymphoid cell (ILC) compartment in endstage lung diseases

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ILCs act as cytokine producers in first-line mucosal defense. We have previously shown that ILC-derived cytokines contribute to shaping of the tissue environment in endstage lungs. Here, we provide an in-depth characterization of the ILC compartment in lungs from patients with endstage cystic fibrosis (CF), COPD/ lung emphysema (EM), and idiopathic pulmonary fibrosis (FI).

We found type 3 ILCs to dominate the ILC compartment in these disease entities, accounting for 46.9%, 44.5%, and 52.6% of ILCs in CF, EM, and FI, respectively, as compared to 20.8% in healthy lungs. Complementing our previous data, we found that an acute allergic exacerbation increased the frequency of natural cytotoxicity receptor (NCR)-positive ILC3s (mean 0.03% vs. 1.2% of ILCs) and decreased type 2 ILCs by 6.8-fold in the peripheral blood of CF patients compared to healthy controls. Finally, treatment of CF patients with a triple CFTR modulator therapy did not substantially change the blood ILC composition in the first 12 months, suggesting that inflammation-associated changes in the ILC compartment may take prolonged periods of time to revert.

Altogether, we show that chronic lung diseases are characterized by major changes in the pulmonary and peripheral ILC compartment that may be valuable indicators of tissue inflammation.

Abstract No. 208

A Drosophila model to study the development and treatment of Cystic Fibrosis

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Cystic fibrosis (CF) is an inherited disease of the CFTR gene and results in an ion imbalance. As a result of this imbalance, the main clinical manifestations are obstructions of the respiratory epithelium of the lungs and the absorptive epithelium of the intestines, particularly due to increased mucus production. Even today, the life expectancy of CF patients is significantly shorter than that of healthy people.
To develop alternative, effective and easy-to-implement therapies, simple yet meaningful disease models are essential. In recent years, *Drosophila* has become a suitable and well-known model for many epithelial diseases. Therefore, in this study, we focus on (i) enhanced mucus production by $CFTR^{KD}$ in fly intestinal enterocytes and (ii) the investigation of two specific mucus-producing genes.
First, we tested different staining methods to detect the mucus layer in the $CFTR^{KD}$ mutants, therewith enabling a quantitative, disease-related read-out. Fluorescence images with lectin staining using WGA showed a massive increase in mucus formation in the intestinal lumen compared to control flies. In addition, we used TCEP, a non-cell permeable disulfide bond-reducing agent, and were able to show a significant reduction in mucus amount after drug administration.
We also used the CRISPR-Cas9 system to generate new fly lines to identify the specific role of the *Mur29B* and *Muc68D* genes in mucus formation. To further validate the mucus composition and the amount of secreted mucus in the generated fly lines, we raised specific antibodies against these mucins.
All in all, this project could be an addition to currently available CF models, which is quick and easy to use, therewith opening up new avenues of investigation and better treatments for people with cystic fibrosis.

Abstract No. 209

Effects of Elexacaftor/Tezacaftor/Ivacaftor Therapy on Mental Health of Patients with Cystic Fibrosis

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Introduction: The CFTR modulator drug elexacaftor/tezacaftor/ivacaftor (ETI) was shown to improve CFTR function and clinical symptoms in patients with cystic fibrosis (CF) with at least one *F508del* allele. Recently, some case reports suggested potential side effects of ETI on mental health with an increase in depressive symptoms and even suicide attempts in patients with CF. However, the general effects of this triple combination therapy on the mental health status of patients with CF remain largely unknown.
Methods: We, therefore, performed a prospective, observational study in a real-life setting and investigated the relationship between initiation of ETI therapy and changes in mental health in adult patients with CF. We assessed Cystic Fibrosis Questionnaire-Revised (CFQ-R), Patient Health Questionnaire-9 (PHQ-9), Beck's Depression Inventory – Fast Screen (BDI-FS) and Generalized Anxiety Disorder 7-item Scale (GAD-7) at baseline and 8-16 weeks after initiation of ETI.
Results: In total, 70 adult patients with CF with at least one *F508del* allele and a median age of 27.9 years were recruited. After initiation of ETI, the CFQ-R respiratory domain score improved by 27.9 (IQR 5.6 to 47.2; $P < 0.001$). The PHQ-9 score of depressive symptoms decreased by 1.0 (IQR -3.0 to 0.3; $P < 0.05$). The BDI-FS score of depressive symptoms decreased from 1.0 (IQR 0.0 to 2.0) at baseline to 0.0 (IQR 0.0 to 2.0; $P < 0.05$) after initiation of ETI. The GAD-7 score of anxiety symptoms did not change after initiation of ETI compared to baseline (0.0; IQR -2.0. to 0.0; $P = 0.112$).
Conclusion: Initiation of ETI improves symptoms of depression in adult patients with CF with at least one *F508del* allele. However, symptoms of anxiety do not change after short-term therapy with ETI.

Abstract No. 210

Evaluation of TMEM16A as a modifier of the cystic fibrosis lung phenotype utilising CF patient-specific human induced pluripotent stem cells

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Previous studies have suggested that the calcium-activated chloride channel TMEM16A may serve as a pharmacological target to treat the CF lung phenotype. It was hypothesized that modulation of TMEM16A function could improve mucociliary clearance in CF, either by activation and enhancement of chloride transport, or by inhibition and reduction of mucus production to prevent mucus accumulation. However, so far no complex *in vitro* model of CF airway epithelial cells is available to investigate both conditions in more detail. Therefore, this study aims to utilise human induced pluripotent stem cell (hiPSC)-derived airway epithelial cells (iALI cultures) with either TMEM16A knockout ($T16^{KO}$) or TMEM16A(*abc*) overexpression ($T16abc^{OX}$) to elucidate the TMEM16A function in CF.
Targeted gene editing was performed to obtain $T16^{KO}$ and $T16abc^{OX}$ cell lines from WT, CF and gene corrected hiPSCs. After differentiation into airway epithelial cell types, CF iALI cultures presented a typical CF disease phenotype characterised by a reduced CFTR protein expression, impaired CFTR-dependent chloride conductance and strong reduction of the ciliary beating that could be partially rescued by application of CFTR modulator drugs. Furthermore, Ussing chamber measurements verified the functional overexpression of TMEM16A in CF-$T16abc^{OX}$ iALI cultures. Additional analysis of the mucus layer properties were performed to detected phenotypical changes related to TMEM16A function.
Our disease model serves as one of the first hiPSC-based disease models of the CF lung disease and provides a unique approach to study TMEM16A as therapeutic target. Answering the question, whether TMEM16A activation or inhibition represents a proper approach for CF treatment will be critical for the development of novel therapies.

Abstract No. 211

Exhaled nitric oxide (FeNO) and hydrogen peroxide (H2O2) as measures of neutrophilic and eosinophilic inflammation in bronchiectasis patients

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Background: Bronchiectasis is a group of heterogeneous lung diseases characterized by obstruction and chronic inflammation of the lower airways. These inflammatory processes are caused by disturbed mucociliary clearance, chronic inflammation as well as bacterial infection with pathogens such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The validity of FeNO and H2O2 as measures of neutrophilic and eosinophilic inflammation in bronchiectasis patients is only incompletely understood.

Methods: Patients with bronchiectasis seen in the Ludwig-Maximilians University Hospital in Munich, Germany were contacted prospectively. We performed FeNO and H2O2 measurements and induced sputum. Clinical as well as demographic data were extracted from hospital records. We analyzed the association between FeNO and H2O2 levels and levels of neutrophilic inflammation and eosinophilic inflammation, exacerbation frequency and bacterial colonization status via Pearson's correlation and Student's t-test.

Results: We recruited 22 patients with bronchiectasis. 12 (54.5%) of these patients were female with an average age of 53 years. H2O2 levels showed a higher correlation with neutrophil levels in sputum (p=0.086, r=0.49) than in blood (p=0.72, r=-0.086). FeNO levels were not well correlated with eosinophil levels in sputum or blood. There was also a significant association between FeNO and the presence of chronic bacterial colonization (p=0.013) and the presence of exacerbations within the last 12 months (p=0.011).

Conclusion: A decrease of FeNO is associated with the presence of chronic bacterial colonization and exacerbations within the last 12 months. This noninvasive parameter can be used to improve monitoring of patients in clinical routine visits, but needs to be further characterized.

Abstract No. 212

Short- and long-term effects of antibiotic therapy on the mycobiome patterns in patients with cystic fibrosis

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Cystic Fibrosis (CF) is one of the most common inherited diseases, affecting about 100,000 individuals worldwide. In the lungs, CF triggers recurrent lung infections and chronic inflammation, which results in respiratory failure and significant morbidity. There is increasing evidence, that both the fungal and bacterial microbiome may play an important role in the pathogenesis and the progression of the disease. While the bacterial microbiome is already well characterized in CF patients, many aspects of the fungal microbiome remain largely elusive. Recent sequencing approaches suggest that the fungal microbiota present in the airways of CF patients is more complex than previously estimated. So far, only a few studies have investigated the occurrence, pathogenicity and the role of fungi in the polymicrobial infections observed in CF patients.
In our study, we characterized the mycobiome of cystic fibrosis patients (in comparison to healthy subjects) and highlighted treatment-associated changes, including short-term effect of antibiotics on the mycobiome during acute treatment after clinical exacerbation as well as long-term effects by the cumulative intake of antibiotics over a 3-years' time period. Our results revealed that CF patients exhibit significantly higher fungal loads in both stool and nasal lavage samples than the healthy cohort. Particularly in nasal samples, CF patients show a significant increase of *Candida* spp. which correlated with the cumulative antibiotic intake in the previous years of therapy.
Our study emphasizes the potential role of fungi in the pathophysiology of CF and discloses the site-specific effects of antibiotic treatment on fungal colonization patterns in CF-patients.

Contribution of a Disease-spanning Working Group: Microbiome-Metagenome

Abstract No. 213

Intervention-driven microbiome changes andantibiotic resistance gene dissemination in patients with cystic fibrosisIntervention-driven microbiome changes andantibiotic resistance gene dissemination in patients with cystic fibrosis

Tilman Klassert[1,2], Cristina Zubiria-Barrera[1,2], Robert Neubert[1,2], Malena Bos[1,2], Jenny Fiebig[1,2], Dominik Driesch[3], Michael Lorenz[4], Jochen Mainz[5], Hortense Slevogt[1,2]

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Cystic fibrosis (CF) is an autosomal recessive disease which affects over 100,000 people worldwide. It is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR), leading to malfunction of exocrine tissues and severe damage to the respiratory and the digestive tracts. Improved therapeutic approaches for most of the CFTR mutations have significantly increased the life expectancy of CF patients. The frequent antibiotic interventions of these patients, however, represent an important manipulation of the microbial communities in the already biased microbiome structures in different organs.
In this study, we performed a longitudinal analysis of the microbiome in the respiratory tract and the gastrointestinal tract of CF patients. Thereby the patients were divided in two different cohorts depending on disease severity and antibiotic administration (ambulant/*per os* (PO) vs. stationary/*intravenous* (IV)). Microbiome analyses were performed before and after therapeutic interventions on acute exacerbation episodes. In addition, long-term therapeutic effects were monitored using clinical data of the cumulative antibiotics intake recorded for the previous 3 years of therapy. Microbial community structures and detection rates of different ARGs was then analyzed in comparative approaches between patient cohorts. Healthy controls and household relatives were further used as outgroups.
Regression models with different therapeutic variables allowed the identification of specific microbiome and ARG dissemination patterns within the CF cohorts. The findings of this study contribute to a better understanding of the short- and long-term impact of antibiotic interventions on the microbial community structures in different organs of CF patients.

Contribution of a Disease-spanning Working Group: Microbiome-Metagenome

Abstract No. 214

Alternative antibiotic treatment to overcome chronic bacterial infections with multi-resistant pathogens such as Pseudomonas aeruginosa

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Usually, antibiotics are used to treat chronic bacterial respiratory infections. However, with the global antibiotic resistance crisis, this is becoming increasingly difficult. In immune-compromised patients or those with cystic fibrosis (CF) or chronic obstructive pulmonary disease (COPD), multidrug-resistant pathogens such as Pseudomonas aeruginosa pose an enormous threat. This opportunistic pathogen has the distinct ability of rapid genetic adaptation and, associated with resistance development, often makes conventional antibiotic therapy useless.
Instead of costly and labor-intensive development of new antibiotics, the study presented here aims to modify the use of already established antibiotics in alternative treatment regimens using the concept of negative hysteresis. In this approach, two antibiotics are used in a short interval, namely a beta-lactam that primes the bacterium first and secondly an aminoglycoside that exerts the desired effect on the pathogen.
To transfer this concept, which has successfully been tested in vitro[1], into an in vivo model, we intend to use a chronic mouse model in which the bacteria are embedded in agar beads prior to application[2]. Thus, we first performed dose-finding experiments for a multidrug-resistant clinical CF isolate. The approved bacterial dose will then be used to test the two antibiotics individually and in combination, before concluding with comparative testing of the alternative treatment regimen based on the negative hysteresis.
This work is in progress, so currently, we are establishing a clinically apparent infection with the selected clinical isolate for several days.

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Abstract No. 215

Application of single-cell RNA sequencing to characterize transient cell populations and molecular signaling during respiratory differentiation of human pluripotent stem cells

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Complex protocols are developed to differentiate human induced pluripotent stem cells (hiPSC) towards mature respiratory epithelia. However, these protocols reflect human development to a limited extent, and the efficiency of the protocols still needs further improvement. At this point, it is not clear to what extent characteristics of individual cell lines play a role, and how contaminating cell populations influence the differentiation outcome.
Therefore, further exploration of cellular composition and molecular signaling of the cells differentiated according to the established protocol is necessary to reveal contaminating cell types and potential signaling pathways to target for the further improvement of the differentiation protocol.
hiPSCs were differentiated towards early respiratory progenitors according to the established protocol. Single-cell RNA sequencing data obtained on day 12 of differentiation revealed lung progenitors (NKX2.1^{pos}) as one major cell population and a population identified as non-lung cells expressing CDX2 – a posterior endodermal, which potentially represents the contaminating cells. Further analysis of the contaminating cell population revealed a cell cluster demonstrating significant upregulation of lipid and chylomicron synthesis-related intestinal cell markers, which confirms that intestinal cells potentially represent one of the contaminating cell types. Besides the respiratory and intestinal cells, the day 12 experimental data also identified pancreatic, neuroendocrine, proliferative, and apoptotic cell populations. Further targeting against the contaminating cell types would potentially lead to the increase of the proportion of respiratory progenitor (NKX2.1^{pos}) cells and to the improvement of the differentiation outcome, respectively.

Contribution of a Disease-spanning Working Group: Single Cell Analysis

Abstract No. 216

Longitudinal Effects of Elexacaftor/Tezacaftor/Ivacaftor Therapy on Viscoelastic Properties of Sputum in Patients with Cystic Fibrosis

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Background:
CFTR dysfunction leads to highly viscoelastic mucus that impedes mucociliary clearance and facilitates chronic airway infection and inflammation that determine the progression of cystic fibrosis (CF) lung disease. The triple combination CFTR modulator therapy elexacaftor/tezacaftor/ivacaftor (ETI) has been shown to improve lung function in CF patients. However, its effects on sputum viscoelastic properties have not been studied.
Methods:
We performed a prospective observational study and assessed viscoelastic sputum properties from 75 CF patients aged 12 years and older with one or two *F508del* alleles before and at 1, 3 and 12 months after initiation of ETI, and 10 healthy controls (www.clinicaltrials.gov: NCT04732910). The elastic (G’) and viscous (G’’) modulus were determined by oscillatory macrorheological measurements.
Results:
At baseline, CF sputum showed significantly higher G' ($P < 0.001$) and G'' ($P < 0.001$) compared to healthy controls. After one month of ETI treatment, there were no significant changes in G' and G'' compared to baseline. After three months of therapy, ETI significantly decreased G' ($P < 0.01$) and G'' ($P < 0.01$) compared to baseline. These improvements were sustained through 12 months of ETI treatment, but sputum properties remained significantly changed compared to healthy controls.
Conclusion:
ETI therapy improves viscoelastic properties of sputum in CF patients with at least one *F508del* allele over a period of 12 months. However, sputum properties did not reach levels observed in healthy controls.
Acknowledgements:
This work was supported by the German Research Foundation (CRC 1449 – 431232613) and the German Federal Ministry of Education and Research (German Center for Lung Research – 82DZL009B1).

Abstract No. 217

iPSC-derived Macrophages in the Study of Cystic Fibrosis Impaired Innate Immunity

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Cystic fibrosis (CF) is an autosomal recessive disease caused by mutations in the *CFTR* gene and leading to, among other things, increased lung infection susceptibility. For many years, it was thought that the defective CFTR ion channel in airway epithelial cells, leading to mucus accumulation in the lungs was decisive for creating an ideal niche for opportunistic pathogens, such as *Pseudomonas aeruginosa,* to colonize CF lungs. However, recently it has been shown that CF alveolar macrophages, forming the first line of defense against airborne bacteria are intrinsically defective in their bactericidal function.
In the absence of established CF macrophage lines, an alternative source is needed to further evaluate the impact of CFTR in these cells. We therefore propose the use of induced pluripotent stem cells (iPSCs) from CF patients and primitive macrophages (iMacs) derived thereof. IMacs can be generated on demand, in a standardized and scalable manner.
For the project, human iPSCs from a p.Phe508del CF patient and a healthy donor (WT) were differentiated into iMacs. Both CF and WT-iMacs presented typical macrophage morphology and surface marker expression. Notably, CF-iMac showed impaired bacterial clearance capacity in comparison to WT-iMacs when exposed to *P. aeruginosa* for 6 hours. Similarly, CF-iMacs showed a delayed capacity to efficiently degrade intracellular bacteria of approximately 1 hour compared to WT-iMacs. Furthermore, in accordance to published research, CF-iMacs showed a higher lysosomal pH and a potentially impaired ROS production mechanism.
With our standardized cell production platform, we are able to provide primitive macrophages from CF patients, which are an ideal model for studying the compromised CF lung innate immunity.

Abstract No. 218

New Treatment Strategy for Pseudomonas aeruginosa Infections in the Cystic Fibrosis Lung

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Multidrug-resistant pathogens are one of the biggest health problems worldwide, especially for people who are particularly susceptible to lung infections, such as cystic fibrosis (CF) patients. Strains of the opportunistic pathogen Pseudomonas aeruginosa, listed by the WHO among the ESKAPE pathogens, are resistant to almost all available antibiotics. In CF patients, chronic P. aeruginosa infections are directly related to progressive lung injury. Therefore, it is of utmost importance to develop a new therapeutic approach for these patients.

Alveolar macrophages, which are the first line of defense against airborne bacteria, are impaired in their bactericidal activity in CF. Our study here investigates the benefit of a therapeutic transfer of fully functional wild type (WT) macrophages into the lungs of CF mice, potentially aimed to develop a cell based immunotherapy to improve the outcome of pulmonary P. aeruginosa infections.

For this pulmonary macrophage transfer (PMT), WT mouse macrophages derived from hematopoietic bone marrow stem cells into the lungs of CF mice before (preventive approach) or after (therapeutic approach) an iatrogenic established P. aeruginosa respiratory infection. Twenty-four hours after infection, lungs were dissected, homogenized, and inflammation parameters and bacterial numbers compared with control animals without PMT. In both treatment strategies, PMT reduced the number of bacteria in the lungs, but only the preventive approach was effective in limiting lung damage caused by the infection, as demonstrated by reduced blood spotting in broncho-alveolar lavage fluid and histologic scoring.

In conclusion, our results are very encouraging with regard to a preventive treatment strategy of applying innate immune cells to the lungs of CF patients.

Abstract No. 219

Nasal nitric oxide increases in patients with cystic fibrosis treated with elexacaftor/tezacaftor/ivacaftor.

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In health, nitric oxide (NO) can be measured in high concentrations in the upper respiratory tract. Nasal NO (nNO) is known to be significantly lower in patients with sinonasal inflammation such as patients with Cystic Fibrosis (CF). In patients with CF treated with elexacaftor/tezacaftor/ivacaftor (ETI), clinical improvement of sinonasal inflammatory symptoms could be observed. We therefore hypothesized that nNO would increase in patients undergoing ETI treatment between a baseline and a second measurement after several months of treatment. Furthermore, we are analyzing possible correlations of nNO with other parameters such as inflammatory markers, lung function and sweat chloride.

33 patients with CF had nNO measurement at baseline before starting ETI and after several months of treatment. 7 patients not undergoing ETI treatment were measured as controls. Preliminary data analyses were conducted for 21 modulated and 5 unmodulated patients with paired measurements.

At baseline and under ETI therapy, nNO had a mean value of 218.9 (SD: 113.53) ppb and 407.06 (SD: 158.58) ppb respectively ($p < 0.001$). For unmodulated patients nNO was 215.17 (SD: 82.22) ppb and 192.64 (SD: 96.77) ppb respectively ($p=0.871$). Analysis for all patients and correlation analysis for inflammatory markers from nasal lavages, lung function testing and sweat chloride is currently ongoing.

In patients with CF undergoing ETI treatment, the nNO value significantly increased after several months of treatment in comparison to baseline whereas in unmodulated patients there was no significant difference between the two measurements. This suggests that nNO is a potential non-invasive biomarker to examine sinonasal inflammatory disease and supports the observation of clinical improvement in these patients.

Abstract No. 220

Longitudinal variation of nasal nitric oxide in patients with Primary Ciliary Dyskinesia and healthy controls

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Introduction
PCD is a rare genetic disorder affecting several organs due to malfunctioning of motile cilia. Nasal nitric oxide (nNO) is significantly decreased in PCD and part of the diagnostic process. However, the longitudinal variation of nNO levels remains unknown.
Aims and objectives
The aim of our study was to investigate the longitudinal variation of nNO levels in patients with PCD and healthy controls.
Method
NNO was measured using the Ecomedics device Analyzer CLD 88sp. 20 patients with PCD and 20 healthy controls (0-64 years) with at least four nNO measurements in one year were included. The measurements were comprised of three breathing manoeuvres - tidal breathing (TB), breath hold (BH) and exhalation against resistance (ER). Data were analysed regarding the coefficient of variation between the measurements and differences between the two groups.
Result
For patients with PCD TB, BH and ER measurement had an average variation of 32.16% (standard deviation (SD): 11.97); 29.45% (SD: 15.04) and 29.88% (SD: 14.82) respectively. Healthy controls had an average variation in TB, BH and ER measurement of 22.59% (SD: 11.97); 18.7% (SD: 11.2) and 19.47% (SD: 9.73) respectively. The difference of the variation between both groups was significant for TB (p=0.015), BH (p=0.043) and ER (p=0.023) measurements.
Conclusion
Our data show that intraindividual nNO values were more variable over the course of one year in PCD patients than in healthy controls but the extent of the variation was moderate. This data supports further investigation of nasal NO as a possible non-invasive quantitative biomarker of disease activity and response to future causative therapy for patients with PCD.

Abstract No. 221

Diagnostic value of multiple-breath washout in patients with chronic productive cough

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Introduction:
Patients with chronic productive cough (CPC) are often referred to tertiary centers to differentiate common causes such as asthma or protracted bacterial bronchitis from rare causes like cystic fibrosis (CF) or primary ciliary dyskinesia (PCD).
Aims and objectives:
The aim of this study was to examine the additional diagnostic value of the lung clearance index (LCI) derived by multiple-breath washout (MBW) as a sensitive measure of lung ventilation homogeneity in comparison with spirometry for differentiation of diseases causing CPC.
Methods:
In addition to our standard diagnostic algorithm including clinical assessment, sweat chloride test, nasal nitric oxide measurement and spirometry we performed nitrogen MBW in 98 patients (age range 2 to 79 years, mean age 21.7 ± 18.0 years) with CPC (COPD excluded). For validation we performed this extended algorithm also in patients with CF (n = 14), PCD (n = 37) and asthma (n = 10) as well as healthy controls (HC) (n = 70).
Results:
Patients with CPC have a higher LCI than HC (8.32 vs. 6.78, $p<0.001$). However, the LCI is able to distinguish between patients with CPC of other causes and patients with PCD or CF (ROC-analysis: AUC 0.70 or 0.66, threshold of LCI 7.05 or 7.02, sensitivity 91.9% or 85.7%, specificity 55.2% or 55.2%, respectively), whereas spirometry is not able to differ these groups (AUC: 0.47 or 0.48, threshold of FEV1 82.2% or 84.8%, sensitivity 50.0% or 62.5%, specificity 58.3% or 53.3%, respectively).
Conclusions:
Due to the high sensitivity of LCI in contrast to FEV1 to distinguish the inherited muco-obstructive diseases CF and PCD from CPC of other genesis, we recommend including the LCI into the diagnostic algorithm for CPC

Abstract No. 222

Microbial metagenomics in health and bronchiectasis

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Research question: Do specific airway metagenome profiles exist among healthy individuals and people with bronchiectasis?
Objective: The global objective of our ongoing studies is to define microbial dysbiosis in chronic lung disease. The extraction of microbial signatures in health and lung disease allows a characterization of microbial communities distinguishing dysbiosis and balanced states.
Methods: We performed whole genome shotgun sequencing of sputum samples from healthy people (n=156) and people with bronchiectasis (n=107). Fragment libraries were sequenced on an Illumina NextSeq system. Quality filtering and alignment of the short reads to a reference database consisting of bacterial, fungal and viral genomes was performed by our in-house developed pipeline Wochenende [1], which additionally normalized the read counts.
Conclusion: The individual metagenomes of people with bronchiectasis clustered by the absence or presence of *Haemophilus influenzae* and *Pseudomonas aeruginosa*. The individual patterns of commensals differed from that of the average healthy control, even in the absence of any pathogen. Common inhabitants of the respiratory tract of healthy subjects including *Neisseria subflava* and *Fusobacterium periodonticum* were rarely detectable in bronchiectasis. Thus, the suppression of indicator commensal species is diagnostic for microbial dysbiosis in people with bronchiectasis.

Contribution of a Disease-spanning Working Group: Microbiome-Metagenome

References:
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Abstract No. 223

Use of primary nasal epithelial cells for personalized CFTR modulator therapy for G85E and N1303K homozygous patients with cystic fibrosis

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Background: CFTR modulator therapy with elexacaftor/tezacaftor/ivacaftor (ETI) is not approved in Europe for individuals with cystic fibrosis (CF) who are homozygous for *G85E* or *N1303K CFTR*. In addition, data on therapeutic benefit of ETI therapy in these patients remain limited.

Objectives: The goal of this study was to test the effects of ETI therapy on CFTR function in primary human nasal epithelial cultures derived from two patients homozygous for either the *G85E* or *N1303K CFTR* mutation in vitro, and correlate findings with real-world clinical response.

Methods: Primary human nasal epithelial cultures (pHNECs) from a 15-year-old female (*G85E/G85E*) and a 19-year-old male (*N1303K/N1303K*) with CF were treated with either ETI or vehicle control for 24h, followed by short-circuit current (Isc) measurement in Ussing chambers. In addition, biomarkers of CFTR function, i.e. sweat chloride concentration, nasal potential difference, and intestinal current measurements, along with clinical parameters, i.e. spirometry and multiple-breath washout measurements, were assessed at baseline and one month after starting ETI therapy.

Results: ETI treatment significantly increased CFTR-mediated Isc in both G85E/G85E and N1303K/N1303K pHNECs to ~11% of normal CFTR function. Moreover, all CFTR biomarkers improved after ETI therapy in both patients, coupled with improvements in clinical parameters.

Conclusions: Our results support that pHNECs are predictive of clinical response of rare *CFTR* mutations to ETI therapy. We suggest that the sequential use of in vitro testing of CFTR function in pHNECs and in vivo testing using CFTR biomarkers with clinical outcome measures is a promising approach to facilitate access to therapy for patients carrying unapproved *CFTR* mutations responsive to ETI.

Abstract No. 224

Longitudinal Effects of Elexacaftor/Tezacaftor/Ivacaftor Therapy on Airway Infection and Inflammation in Patients with Cystic Fibrosis

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Background

The triple combination CFTR modulator therapy with elexacaftor/tezacaftor/ivacaftor (ETI) improves CFTR function and abnormalities in lung morphology in CF patients with at least one *F508del* allele. However, the longitudinal effects of ETI on the airway microbiome and chronic airway inflammation have not been reported. The aim of this study was therefore to examine the effects of ETI on the airway microbiome, inflammation and proteome in CF patients with one or two *F508del* alleles through the first 12 months of therapy.

Methods

We performed a prospective observational study assessing the sputum microbiome, inflammation markers and proteome before and at 1, 3 and 12 months after ETI initiation (www.clinicaltrials.gov: NCT04732910).

Results

75 patients with CF and at least one *F508del* allele and 10 healthy controls were enrolled in this study. ETI increased the microbiome α-diversity ($P<0.001$) at 3 months, which was associated with a reduced relative abundance of *Pseudomonas aeruginosa* ($P<0.05$), and decreased sputum IL-1β and IL-8 levels ($P<0.01$). Further, ETI reduced free neutrophil elastase activity ($P<0.001$) and shifted the protease/antiprotease balance and the sputum proteome in CF patients towards healthy condition. These improvements were sustained over 12 months, however, residual abnormalities in CF patients remained compared to healthy controls.

Conclusions

ETI improves the airway microbiome diversity and chronic airway inflammation in CF patients with at least one *F508del* allele over a period of 12 months, however, without reaching levels close to healthy.

Acknowledgements

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Abstract No. 225

Spirometric and anthropometric improvements in response to elexacaftor/tezacaftor/ivacaftor depend on age and lung disease severity

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Introduction: Triple combination CFTR-modulator therapy with elexacaftor/ tezacaftor/ ivacaftor (ETI) was introduced in 08/20 in Germany for people with CF (pwCF) >12 years (yrs.) of age and 06/21 for pwCF >6 years of age. In this single-center study, we analyzed longitudinal data on ppFEV1 and BMI for 12 months (mo.) after initiation of ETI by linear mixed models and regression analyses to identify age- and severity-dependent determinants of response to ETI.
Methods: We obtained data of 42 children >6-11 yrs., 41 adolescents ≥12-17 yrs. and 143 adults with spirometry and anthropometry prior to ETI and 3 and 12 mo. after ETI initiation. Data were stratified according to age-groups and age-specific lung function impairment (ppFEV1 ≤25%perc. (<P25), 26-74%perc. (P50), ≥75%perc. (>P75).
Results: Adolescents and children with more severe lung disease prior to ETI (ppFEV1 ≤P25) showed higher improvements in lung function compared to adults in this severity group (+18.5 vs. +7.5; p=0.002 after 3 mo. and +13.8 vs. +7.2; p=0.012 after 12 mo. of ETI therapy for >12-17yrs. and +19.83 vs. +7.5; p= 0.007 after 3 mo. for children >6-117 yrs.). In all age groups, participants with more severe lung disease (ppFEV1 ≤P25) showed higher BMI gains compared to those with medium or good lung function (ppFEV1 P50- >P75). Regression analyses identified age as a predictive factor for FEV1 increase at 3 mo. after ETI initiation and age and ppFEV1 at ETI initiation as a predictive factor for FEV1 increase 12 mo. after ETI start.
Discussion: We report initial data, which suggest that clinical response towards ETI depends on age and lung disease severity prior to ETI initiation, which argue for early initiation of ETI.

Abstract No. 226

Survival in cystic fibrosis is dependent on TNFR1 genotype and TNFR1 transcript processing in epithelial cells

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In recent years, modifying genes of CF have been described using large and complex multicentric studies, compiling data on more than 1000 CF patients and using start-of-the-art computational methods. However, the gene encoding the tumor necrosis factor alpha receptor TNFR1, one CF modifying gene described previously within small, albeit carefully characterized patient subsamples, was not replicated in the aforementioned large GWAS studies.

We have analysed the association of TNFR1 genotypes with patient survival as described previously for another CF modifying gene, we have revisited the previously described association the CFTR-mediated basic defect in a replication cohort for that particular endophenotype, we have studied TNFR1 transcript variants in model epithelial cell lines and finally, undertook a genotype-phenotype association study in primary airway epithelia to correlate TNFR1 genotype with TNFR1 transcript repertoire.

Association of TNFR1 genotype with manifestation of CFTR-mediated basic defect could be replicated (n = 25, P = 0.04) Patient survival differed comparing carriers of contrasting TNFR1 genotype (n = 60, P = 0.002). Presence of a previously described TNFR1 transcript variant in primary airway epithelia was associated with TNFR1 genotype (n = 64, P = 0.04).

Our data suggest that genetic variants within TNFR1 intron 1 determine aberrant splicing of TNFR1 transcripts in epithelial, but not in lymphoid cells. Our observations furthermore show that among patients born in the mid-1970s to mid-1980s, carriers of contrasting TNFR1 genotypes differ in mean patient survival by more than a decade. We conclude from our data that TNFR1 is a major modifying gene in cystic fibrosis which might easily be missed due to a recruitment bias in survivor populations.

Abstract No. 227

Determinants of survival in a European cystic fibrosis twin and sibling population

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The European CF Twin and Sibling study EUCFTSib [1,2] has collected data and biomaterials since the mid-1990s. Now, data on "time of death" and/or "time of lung transplant" has been retrieved for participants in EUCFTSib.
We wanted to know whether risk genotypes at 10 gene modifier loci for which we can annotate risk and benign alleles [1,3–5] predispose to worse outcome. We have observed patient survival to be dependent on CF modifier genotype for TNFR1 (Pmin < 0.001).
Next, we have asked whether serology data, measured in the 1990s, are correlated with patient survival among from 45 F508del-CFTR homozygous sibling pairs. Patients who were still alive at the end of the observation period had lower IgG (P = 0.0000667), IgA (P = 0.00071), IgG1 (P = 0.0016), loer IgG2 (P = 0.0000025), IgG4 (P = 0.0072) and sIgG-specific-for-Aspergillus (P = 0.00013). Of note, immunoglobulin levels from 30 monozygous twins were similar and seemed independent on survival status of the twins in comparison to a sibling subset of similar size.
Finally, we have asked whether CF modifier genes with a well-defined role in immunology are associated with serology. Absence of carriership for risk alleles at IL17A/IL17F locus was associated with higher IgG4 levels (P=0.017) and absence for risk alleles at STAT3Sat was associated with higher IgG1 levels (P=0.0013).
Among the tested CF modifier genes is this study, TNFR1, IL17A/F and STAT3 are suggested as relevant genes for prediction of disease prognosis and patient survival. Targeted treatment of inherited risk determined by CF modifier genes is within reach as therapeutics are available that specifically intervene with cytokine and interleukin pathways.

Abstract No. 228

The relationship of the corrected N2-lung clearance index with the SF6-lung clearance index and magnetic resonance imaging

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Introduction: Multiple-breath washout (MBW) is sensitive to detect lung ventilation homogeneity in children with cystic fibrosis (CF) lung disease. A recently described sensor error in the commonly used spiroware software used with the Exhalyzer D leads to a change in nitrogen-derived MBW and requires reevalution of its impact on previous results and its clinical implication.
Methods: 20 healthy controls and 31 infants with CF underwent consecutive sulfur hexafluoride (SF_6)- and nitrogen(N_2)-MBW with a chest magnetic resonance imaging (MRI) on the same day. Previously published N_2-MBW results were reanalyzed with the corrected software 3.3.1 and compared to SF_6-LCI and findings in chest MRI.
Results: Correction of the N_2-derived lung clearance index (LCI_{cor}) led to a significant decrease of the LCIcor in healthy and children with CF compared to previous results (LCI_{prev}) (P=0.001). Despite of more similar SF_6- and N_2-LCI_{cor}, the N_2-LCI_{cor} remained significantly higher than the SF_6-LCI (P<0.01), but correlated significantly with it (P=0.03). The upper limit of normal of N_2-LCI_{cor} based on the healthy group decreased and changed the health-disease classification in a third of the children with CF. N_2-LCI_{cor} correlated well with the MRI scores (P<0.01-0.001). A detailed analysis of N_2-LCI_{cor} and MRI is currently ongoing and will be available at the conference.
Conclusion: The sensor error correction has entailed a significantly decreased N_2-LCI_{cor}. MBW results with N_2 and SF_6 as tracer gases remain not interchangeable, but key findings of previous observational studies can be confirmed.

Abstract No. 229

Development of an in vitro disease model for Primary Ciliary Dyskinesia utilizing patient-specific human induced pluripotent stem cells

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Primary ciliary dyskinesia (PCD) is a rare genetic disorder caused by mutations in different genes associated with motile cilia biogenesis or function. Motile cilia dysfunction leads to diminished mucociliary clearance of pathogens in the respiratory tract, leading to chronic airway infections causing progressive lung damage. Current approaches to treat PCD are only symptomatic, showing the urgent need for innovative models to increase understanding of disease mechanisms as well as development of new curative therapeutic options. So far, in vitro modelling as well as drug-screening approaches for PCD were only performed using primary respiratory cells from PCD patients or immortalized non-airway cell lines, which harbor substantial limitations in terms of accessibility, expandability, genetic manipulability or recapitulation of diseased functional phenotype, respectively. Hence, we developed an *in vitro* model for PCD, based on patient specific human induced pluripotent stem cells (hiPSC).

hiPSCs from PCD patients and healthy controls were successfully differentiated towards airway epithelium in air-liquid interface (ALI) cultures showing expression of typical airway epithelial markers (AcTub, MUC5AC, P63, KRT5, CCSP). By applying transmission electron microscopy, immunofluorescence staining, ciliary beat frequency and mucociliary transport measurements, we could demonstrate that ciliated respiratory epithelial cells derived from two PCD patient specific hiPSC lines carrying mutations in *DNAH5* and *NME5*, respectively, recapitulate the diseased phenotype on molecular and functional level. Currently, our model is being used for further elucidating disease underlying mechanisms as well as drug testing for PCD therapy.

Abstract No. 230

Optimizing Lung Segmentation on chest X-Ray in Pediatric Cystic Fibrosis Patients Through Dataset Analysis and Deep Learning

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Objectives:
Cystic fibrosis (CF) is a life-threatening genetic disorder that significantly affects the lungs, requiring effective diagnosis and continuous monitoring. X-ray imaging remains a crucial method for diagnosing and tracking the progression of CF because of wide availability and low radiation dose.

Methods and Materials:
In this study, we have collected 674 X-ray images of CF patients, accompanied by their magnetic resonance tomography (MRT) scores. Our analysis focused on the severity of the disease and the age distribution of the patients. The results illustrate the relationship between the severity of CF and time, with a significant portion of our dataset comprising pediatric patients under 6 years of age.

Results:
We observed that existing datasets used for training segmentation neural networks contain few or no pediatric X-ray images contrary to our database situation, resulting in subpar performance when applied to the pediatric group. In response, we implemented methods to improve segmentation performance by incorporating more pediatric X-ray images into the training dataset. As a result, the refined neural network demonstrated enhanced performance in pediatric patients, particularly those under 6 years of age.

Conclusion:
In conclusion, the primary reason affecting the segmentation of images in young children is the unclear boundary between the left lung and the abdomen. Enhancing the contrast of the images through suitable contrast adjustment techniques can improve the segmentation accuracy for some images. However, incorporating pediatric images into the training set is a more effective approach to improve the segmentation.

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Abstract No. 301

Cystic fibrosis transmembrane conductance regulator modulators attenuate platelet activation and aggregation in blood of healthy donors and COVID-19 patients

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Mutations in cystic fibrosis transmembrane conductance regulator (CFTR) cause the clinical presentation of cystic fibrosis (CF), but have recently also been shown to cause agonist-induced platelet hyperactivation. These findings are reminiscent of platelets from SARS-CoV-2 infected patients since thromboembolic complications represent hallmarks of severe COVID-19. CFTR modulators have recently been introduced as a treatment for patients with various CFTR mutations, but have also been reported to effectively enhance channel function of wild type CFTR. We therefore postulated that CFTR modulators may exert anticoagulant effects on platelets of healthy donors (HD) and COVID-19 patients.
Pre-treatment with the CFTR modulator ivacaftor significantly reduced platelet agonist-induced CD62p/CD63 expression, Ca^{2+}-mobilization, aggregation, and adhesion of platelets from HDs. In blood of COVID-19 patients, platelet activation correlates with disease severity, as demonstrated by a significant increase in the proportion of $CD62p^{+}$ platelets from acute COVID-19 patients relative to HDs. Retrospective analysis of clinical data from CF patients with COVID-19 receiving CFTR modulator therapy in comparison to an untreated cohort revealed that CFTR modulators reduced the relative risk to suffer thromboembolism-associated cardiovascular events. E*x vivo* pre-treatment of platelets of acute COVID-19 patients with ivacaftor reduced Ca^{2+} mobilization, adhesion, and aggregation of platelets.

Our results demonstrate an anticoagulant effect of CFTR modulators on platelets of HDs and severe COVID-19 patients and thus, suggest CFTR modulators as promising strategy to reduce the risk of thrombotic events in the clinical management of COVID-19 and similar pro-thrombotic disease states.

Abstract No. 302

Human induced pluripotent stem cell (hiPSC-) -derived lung organoids for disease modelling

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Lung organoid models derived from mouse and human stem cells have become a powerful tool to study lung development and disease including modelling infections and drug screening. Here, we describe a bud-tip lung organoid (BTO) model derived from human induced pluripotent stem cells (hiPSC). To generate hiPSC derived-BTO, the pluripotency of the hiPSC lines was verified by the expression of Nanog, Oct4, TRA-1-81, TRA-1-60, SSEA-3, SSEA-4 by flow cytometric analysis, Western blot, and immunofluorescence. Subsequently, hiPSC were differentiated into endoderm followed by generation of NKX2.1^{+} ventral-anterior foregut endoderm. After 4-6 weeks, these spheroids formed branched structures containing differentiated cells, such as MUC5A+ goblet cells, SFTPB+ alveolar epithelial cells type 2 and SCGB1A1 club cells. BTOs represent a tool to model infection by supporting the viral replication of H5N1 and SARS-CoV2/München-1.2/2020/984 viruses. Notably, infection with SARS-CoV2 for up to 72 hours showed a time-dependent increase in both virus titers and mRNA expression of SARS-CoV-2 nucleocapsid (N) and envelope protein. The expression of SARS-CoV-2 N-protein was also determined by immunofluorescence. To increase the complexity of the model, hiPSC-derived endothelial cells and endothelial networks from blood vessel organoids were co-cultured with BTOs, resulting in successful parenchymal-vascular communication, assessed by immunofluorescence. In summary, we were able to successfully generate a human lung organoid model based on hiPSC, with the possibility to further develop its cellular and morphogenic complexity to model diseases (e.g. infections), cell- and gene-specific responses by hiPSC genome editing (under development) and personalized approaches using patient-derived hiPSC.

Abstract No. 303

Patient-derived alveolar organoid disease model to study gene editing therapy in STAT3-hyper IgE syndrome

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Research question: STAT3-hyper IgE syndrome (STAT3-HIES) is a multisystem disease, caused by heterozygous dominant-negative mutations in *STAT3.* Respiratory complications particularly impact the quality of life of patients. We aim to tackle the current lack of therapy to regenerate the respiratory epithelium. As alveolar epithelial type 2 cells (AEC2) play a major role in regeneration as stem cells of the alveolus, we hypothesize that correcting the disease-causing mutations in patients' AEC2 would rescue alveolar tissue repair mechanisms.
Objectives: We aim to study the therapeutic effect of gene repair in STAT3-HIES patient-derived alveolar organoids.
Methods and Results: Induced pluripotent stem cells (iPSCs) were reprogrammed from STAT3-HIES patient fibroblasts by transient transgenic overexpression of reprogramming factors. The *STAT3* p.R382W mutation was corrected in STAT3-HIES patient iPSCs with CRISPR/Cas9-mediated adenine base editing (see Eberherr et al.[1]). Corrected and uncorrected patient iPSCs were successfully differentiated into iAEC2. Immunofluorescence staining of surfactant protein C and NK2 homeobox 1 (NKX2.1) confirmed alveolar epithelial cell identity. RT-qPCR analysis showed increased expression of the STAT3 target gene *SOCS3* after stimulation with IL-6 and soluble IL-6 receptor and quantification of cell numbers per organoid indicated a higher proliferation capacity of corrected iAEC2 when compared to untreated iAEC2.
Conclusion: Alveolar organoids are a suitable human model for studying the pathophysiology of STAT3-HIES and gene repair therapy. Preliminary results point toward a positive effect of gene repair to rescue AEC2 function and thus to promote alveolar tissue recovery after injury.

Contribution of a Disease-spanning Working Group: Lung Environment Interaction

References:
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Abstract No. 304

Neutrophil efferocytosis locks alveolar macrophages in a pro-resolution phenotype at the cost of bacterial control

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RATIONALE
Resolution of lung injuries is vital to maintain gas-exchange. Concurrently, there is an increased risk of bacterial superinfections. Alveolar macrophages are crucial in the control of inflammation and bacterial pathogens. However, how the control of these putatively opposing macrophage effector functions is directly connected remains incompletely understood.
METHODS
Aspiration pneumonitis was induced by instillation of HCl i.t. into mice. Flow-sorted AMs were subjected to bulk RNA sequencing. AMs were harvested by bronchoalveolar lavage and assessed for bactericidal properties, cytokine secretion, and metabolic properties ex vivo. Oxygen consumption rate and glycolysis were measured using an extracellular flux analyzer.
RESULTS
Based on transcriptomic and functional analyses, we identified a transient incapacity of AMs to react to bacteria during resolution of inflammation. Importantly, efferocytosis of neutrophils impaired bactericidal properties of AMs. Mechanistically, upregulation of UCP2 by neutrophil-derived myeloperoxidase (MPO) fueled glutamine-dependent oxidative phosphorylation, which precluded an increase in the mitochondrial membrane potential, and, consecutively, a release of mitochondrial reactive oxygen species in response to bacteria. Inhibition of UCP2, or canonical glutaminolysis, as well as genetic deletion experiments, confirmed our findings in secondary bacterial infections after acid aspiration and influenza pneumonia. Human AMs and other murine macrophages were functionally similarly altered by MPO.
CONCLUSIONS
Increased UCP2 expression consecutive to neutrophil efferocytosis represents a conserved immunometabolic switch that restricts the plasticity of TRMs to prioritize resolution of inflammation over innate immunity to bacteria.

Abstract No. 305

The effect of surfactant protein B (SP-B) deficiency on alveolar micromechanics

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Surfactant protein B (SP-B) is one of the hydrophobic surfactant proteins, which is mainly responsible for reducing the surface tension inside the alveoli. Earlier studies revealed the SP-B-content in the bronchoalveolar lavage (BAL) and thus in the alveolar compartment of the lung to be significantly reduced even before a patient shows symptoms of respiratory failure while receiving mechanical ventilation in terms of the ARDS. It remains unclear, whether this relationship is causal or not. Nonetheless, the SP-B deficiency represents a characteristic of the lung that increases the risk to exacerbate and cause ventilation induced lung injury (VILI).
The aim of this study was to investigate the effect of decreasing SP-B levels in the lung on the alveolar micromechanics and hence the vulnerability to develop VILI. A conditional SP-B knockout mouse model expressing SP-B under control of a doxycycline dependent promotor was used. Two days after withdrawal of doxycycline, BAL showed reduced SP-B and increased albumin levels. Lung mechanical properties were measured during mechanical ventilation. The time point of the investigation represents a preclinical state with subtle anomalies in lung mechanics: Tissue elastance was slightly increased and quasi-static pressure-volume loops demonstrated a left shift of the upper infliction point: lungs entered the non-linear portion of the pressure-volume relationship already at lower pressures during inspiration. To understand the micromechanical abnormality responsible for the observed changes in the PV-loops, which might be due to a failure in recruitability of distal airspaces e.g. by unpleating of septal walls, lungs were fixed at different airway opening pressures for design-based stereology at light- and electron microscopic level.

Abstract No. 306

Evaluation of treatment response with serial CT in patients with non-tuberculous mycobacterial pulmonary disease

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Objectives: In patients with nontuberculous mycobacterial pulmonary disease (NTM-PD) the response to treatment is evaluated based on microbiological, clinical and radiological data. However, little is known about the dynamics of CT findings. The aim of this study was to evaluate CT changes in NTM-PD in order to define radiological criteria for treatment success.
Methods: Retrospective single centre study. 30 patients with NTM-PD and at least two consecutive CT were included. Scoring for NTM-PD was performed evaluating bronchiectasis, mucus plugging, bronchiolitis, cavities, nodules, and consolidations on an ordinal scale from 0-3. Differences between baseline and follow-up were calculated and patients with/without cultural conversion were compared using the Mann-Whitney U test. For paired comparison of the two consecutive CT scans the Wilcoxon test was used.
Results: Comparing patients with and without culture conversion there were significant differences for bronchiectasis, cavities and the total score. In case of culture conversion, there was a decrease of the total score (0.50 in persistent cultures versus -1.93 with conversion; p=0.011) and cavities (0.00 in persistent cultures versus -0.86 with conversion; p=0.024), whereas bronchiectasis were increasing (0.69 in persistent cultures versus 0.00 with conversion; p=0.016). The Wilcoxon test showed significant differences for bronchiectasis between baseline and follow-up with an increase from 4.25 to 4.94 (p=0.014).
Conclusions: In case of culture conversion, cavities and bronchiolitis decreased in patients with NTM-PD whereas chronic lung changes such as bronchiectasis and nodules remained constant and even increased with persistence of positive cultures.

Abstract No. 307

Sars-Cov-2 infection leads to lasting effect on local lung myeloid compartment

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The COVID-19 outbreak has claimed more than 6.8 million lives to date. The survivors indicate a bleak outlook where a large fraction of the recovered patients report symptoms, such as chest pain, abnormal breathing and myalgia several months after viral clearance, a condition called "Long COVID". A number of studies have reported abnormal gas transfer, decreased diffusion capacity and 6-minute walking distance for these Long COVID patients. These findings indicate an impaired repair mechanism and/or dysregulated immune response in the lung and call for further investigation of the molecular mechanism underlying Long COVID symptoms.
We hypothesize a disrupted local innate immunity in the lungs of COVID survivors that may sensitize towards chronic lung disease. To test this, we compared publicly available single-cell transcriptomics data from the BALF of COVID-19 patients up to 98 days after symptom onset with healthy controls, smokers, and COPD patients. Using K-nearest neighbor classification, we identified an alveolar macrophage cluster from COVID-19 patients at 75 and 98 days after symptoms onset that was transcriptomically similar to macrophages from smokers, revealing a non-homeostatic state. Differential gene expression (DE) analysis against healthy controls revealed 146 DE genes. Among the top upregulated was *GDF15*, a known predictor of COPD severity. Pathway analysis indicated metabolic alterations in oxidative phosphorylation, cellular respiration and response to stress which suggests a shift away from homeostasis. Additionally, *TNFα* mediated signaling and *MTORC1* signaling were enriched in the upregulated genes. Taken together, our analysis revealed that persisting abnormalities in alveolar macrophages may make survivors susceptible to lung diseases such as COPD.

Contribution of a Disease-spanning Working Group: Lung Environment Interaction

Abstract No. 308

Outer membrane vesicle of *Klebsiella pneumoniae* decrease bactericidal properties of alveolar macrophages

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Introduction: Alveolar Macrophages (AMs) are the sentinel cells in the lung, which clear bacteria and initiate inflammation. Gram-negative bacteria release outer membrane vesicles (OMVs) and antibiotics increase OMV production. OMVs contain different types of cargo such as proteins and lipids. As colonization with *Klebsiella pneumoniae (K. pneumoniae)* constitutes a risk factor for infection, we hypothesized that OMVs of *K. pneumoniae* might alter bactericidal properties of AMs to facilitate pneumonia.

Methods: *K. pneumoniae* were cultured *in vitro*, treated with different antibiotics, and the secreted OMVs were isolated. Murine AMs were harvested by bronchoalveolar lavage and treated with OMVs. Bactericidal properties were assessed in AMs infected with viable *K. pneumoniae ex vivo*. Reactive oxygen species (ROS) were quantified by flow cytometry. Cytokines were measured using a multiplex bead-based assay.
Results: Preincubation with OMVs significantly decreased the killing capacity of AMs. In line, intratracheal instillation of OMVs facilitated bacterial outgrowth in a subsequent infection with *K. pneumoniae*. Whereas, OMVs did not alter cytosolic ROS production, they abrogated mitochondrial (mt) ROS release and decreased cellular respiration in AMs in response to *K. pneumoniae*. Specifically, OMVs isolated from *K. pneumoniae* treated with meropenem or piperacillin/tazobactam had the strongest effect. Inactivation of proteins and not DNA or RNA in permeabilized OMVs abrogated inhibition of mtROS release upon bacterial encounter
Conclusion: In summary, we found that OMVs of *K. pneumoniae* dampen the killing capacity of AMs.Therefore, we suggest that OMVs might facilitate the transition from bacterial colonization to infection in the lung by decreasing bactericidal properties of AMs

Abstract No. 309

Increased susceptibility to Pseudomonas aeruginosa pneumonia in overventilated lungs is caused by airspace acidification

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Ventilator-associated pneumonia (VAP) is the most frequent nosocomial infection in critically ill patients. Local pH fluctuations influence bacterial growth. Whether airway acidification contributes to the pathogenesis and pathophysiology of *Pseudomonas aeruginosa* (PA)-induced VAP is largely unknown.

To investigate the role and mechanisms of airspace acidification by mechanical ventilation (MV) in PA-induced VAP, C57BL/6J mice were subjected to high (HVt:34mL/kg) or low (LVt:9mL/kg) tidal volume MV for 4h. PA was administered via the tracheal tube and the animals were allowed to recover from sedation and breathe spontaneously for 24 hours after extubation. Fluorescence microscopy was applied to determine alveolar pH in *ex vivo* perfused and ventilated murine lungs, and bacterial growth and adhesion on cyclically stretched A549 and human airway epithelial cells. Our results showed that the HVt mice showed increased alveolo-capillary permeability, elevated lung and blood leukocyte counts, and a higher PA load in lungs and extrapulmonary organs as compared to LVt controls. HVt MV induced acidification of alveolar lining fluid (ALF) in lungs was found to be correlated with decreased pulmonary expression of Na+/H+exchanger1 (NHE1). Inhibition of NHE1 enhanced growth of PA *in vitro* on epithelial cells and increased pulmonary bacterial loads in LVt-MV mice *in vivo*.

In our novel murine VAP model, HVt MV induced mild VILI with airway lining fluid acidification and increased susceptibility to PA pneumonia. NHE1 was identified as critical factor for MV-induced airspace acidification, and thus as possible target to combat PA-VAP.

Abstract No. 310

Prone position ventilation decreased the susceptibility to murine Pseudomonas aeruginosa pneumonia

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Ventilator-associated pneumonia (VAP) caused by Pseudomonas aeruginosa (PA) is a common intensive care unit-acquired nosocomial infection. Early prone positioning (PP) during mechanical ventilation (MV) has been proven beneficial in severe ARDS patients. The potential effect of PP on the development of VAP is currently unknown.
To investigate the role of PP during MV on PA-VAP severity C57BL/6J mice were subjected to high tidal volume MV for 4h either in supine (SP) or prone position and PA was instilled via the endotracheal tube. Sedation was antagonized and mice breathed spontaneously for 24h after extubation. Lung function was measured at the start and end of MV. Lung permeability, inflammatory responses, histology, and bacterial load in lung, blood, and liver were analyzed 24h post-infection.
PP mice exhibited decreased mean airway pressure and increased lung compliance after 4h MV. PP mice showed significantly decreased lung permeability, reduced BAL and plasma levels of inflammatory mediators, and lower blood neutrophil counts compared to infected SP mice. Pulmonary and extra-pulmonary bacterial loads were significantly lower in mice subjected to PP than SP. Histopathologic analysis showed significantly attenuated neutrophil infiltration and lung injury in infected PP mice compared to the SP mice. Analysis of dorsal and ventral lung sections revealed a significant reduction of ventral overinflation and neutrophil infiltration in PP mice.
In conclusion, our novel murine VAP model, prone positioning prevented worsening of lung function due to MV and was associated with decreased susceptibility to PA infection and reduced systemic spread of bacteria.

Abstract No. 311

Human primary alveolar epithelial cells in air-liquid interface transdifferentiate into basal like intermediates

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Due to its large surface area, the alveolar blood-gas barrier presents an attractive route for non-invasive drug delivery. Pharmacokinetic studies across this barrier are traditionally hampered by the limited availability of primary alveolar epithelial cells and limited translatability of cancer-derived cell lines like A549 forming no relevant barriers or NCI-H441 which tend to grow in oligolayers. Of late, human primary alveolar epithelial cells (hPAEpC) have become commercially available. Here, we characterized these cells at a morphological, functional, transcriptional and proteomic level.

Optimal growing conditions for hPAEpC were determined as 8 days at a gas-liquid interface with an initial seeding number of 7,500 cells per cm^2 resulting in a tight cell monolayer as determined by TEER and expression of ZO-1. Ussing chamber recordings detected an amiloride-sensitive short circuit current. Western blot and immunocytochemistry revealed specific markers of alveolar epithelial cells incl. AQP5 and CAV1 for type I and SFTPC and HLA-DR for type II cells, yet marker expression decreased rapidly as a function of passage number. Electron microscopy showed polarized cells with apical tight junctions, microvilli and lamellar bodies in all passages. In single cell transcriptomics, specific alveolar hallmark genes like NKX2.1 or surfactant proteins were absent, while a respiratory epithelial character was confirmed.

We conclude that hPAEpC share morphological and functional characteristics of alveolar epithelial cells without fully replicating their transcriptomic and proteomic expression profile. Barrier function is well represented by these cells, yet specific alveolar epithelial characteristics are rapidly lost at higher passages.

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Abstract No. 312

Role of Macrophage-inducible C-type lectin Mincle in staphylococcal pneumonia in mice

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Staphylococcus aureus (*S. aureus*) is one of the most prevalent pathogens in nosocomial pneumonia. By using WT, Mincle KO and Mincle transgenic (tg) mice, we examined the role of Mincle in lung host defense against *S. aureus*. Two glycolipid variants, Glc-DAG and Glc_2-DAG were purified from *S. aureus*, of which only Glc-DAG triggered reporter cell activation and elicited inflammatory cytokine responses by professional phagocytes *in vitro*. Importantly, such Glc-DAG-induced reporter cell activation was blocked dose-dependently in the presence of Glc_2-DAG. Moreover, as evidenced by proteomic profiling of WT phagocytes, Glc-DAG-induced inflammatory cytokine responses, including IL-1β, were blocked in the presence of Glc_2-DAG, showing that Glc_2-DAG competitively blocks Glc-DAG/Mincle signaling. Collectively, we show that *S. aureus* harbors Glc_2-DAG variants serving as endogenous inhibitors of Mincle-Glc-DAG dependent cytokine responses, most likely to attenuate the process of bacterial pathogen recognition by Mincle. These studies may provide a molecular explanation for the frequently observed chronification of staphylococcal diseases in patients.

Abstract No. 313

Community-acquired pneumonia in adults – results from 20 years of CAPNETZ study

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The network of competence on community-acquired pneumonia (CAP) was established in 2001 by the German Ministry of Research and Education. Since initiation of the CAPNETZ study in 2002 numerous findings have contributed to the current understanding of CAP pathology and epidemiology and could be translated into a continuously improving disease management.
In 2023, the multicenter, multinational CAPNETZ cohort is over 14 000 patients strong. Associated to this is a variety of clinical data with around 2500 entries per patient recording visits, patient demographics, comorbidities, CAP pathogens, medication, disease outcome, and more. The CAPNETZ dataset as well as biosamples taken during hospitalization constitute the foundation of associated CAP research.
The development of CAP mortality, comorbidities, and epidemiology over the course of 20 years will be presented and the impact of German CAP guidelines on these variables will be demonstrated, for example on CAP medical treatment. The mortality of CAP did not change significantly during the last 20 years. In contrast, the microbiology changed, particularly since the occurrence of the novel SARS-CoV-2 virus.
CAP remains a leading cause of death globally and in Germany. However, advances in biomedical research and technology paralleled by careful analysis of clinical data from the CAPNETZ study will contribute to the reduction of the burden of this disease.

Abstract No. 314

Bacterial vesicles block viral replication in macrophages via TLR4-TRIF-axis

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Gram-negative bacteria naturally secrete nanosized outer membrane vesicles (OMVs), which act as a means of communication. OMV uptake by host cells activates TLR signalling via transported PAMPs. As key resident immune cells, alveolar macrophages are located at the air-tissue interface, where they comprise the first line of defence against inhaled microorganisms and particles. To date, little is known about the interplay between alveolar macrophages and OMVs from pathogenic bacteria. Immune responses to OMVs and underlying mechanisms are still elusive. Here, we examined the response of primary human macrophages to bacterial vesicles (*Legionella pneumophila*, *Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella enterica*, *Streptococcus pneumoniae*) and observed comparable NF-κB activation across all tested vesicles. In contrast, we describe differential type I IFN signalling with prolonged STAT1 phosphorylation and Mx1 induction blocking influenza A virus replication only for *Klebsiella*, *E.coli* and *Salmonella* OMVs. OMV-induced antiviral effects were less pronounced in endotoxin-free *Clear coli* OMVs and Polymyxin-treated OMVs. LPS stimulation did not mimic this antiviral status, while TRIF knockout abrogated it. Importantly, supernatant from OMV-treated macrophages elicited an antiviral response in alveolar epithelial cells (AEC), suggesting OMV-induced intercellular communication. Finally, results were validated in an *ex vivo* lung infection model.
Collectively, *Klebsiella*, *E.coli* and *Salmonella* OMVs induce antiviral immunity in macrophages via TLR4-TRIF signaling, thereby limiting viral replication in macrophages, AECs and lung tissue. These gram-negative bacteria induce antiviral immunity in the lung through OMVs, potentially affecting bacterial and viral coinfection outcome.

Abstract No. 315

Impact of *Klebsiella pneumoniae* outer membrane vesicles on antibiotic resistance

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Klebsiella pneumoniae (*Kp*) is a pathogenic bacterium that can cause pneumonia. The emergence of hypervirulent and multidrug-resistant strains has made *Kp* one of the antibiotic-resistant pathogens prioritized by the WHO. It is essential to understand their mechanisms of pathogenicity. Our study investigates the role of outer membrane vesicles (OMVs), which are nano-sized structures consisting of a lipid bilayer, in the interplay between antibiotics and bacteria. Specifically, we analyze the impact of stress conditions on OMV release and their effect on antibiotic resistance in *Kp*.
Our results indicate that *Kp* releases OMVs of varying size and amount under different stress conditions. We show that exposure to polymyxin B (PB) leads to significant changes in the vesicle composition but is not associated with an upregulation of PB resistance genes. Lipidomics analyses reveal that PB alters the composition of OMVs but not of the source bacteria. Importantly, we demonstrate that OMVs protect *Kp* from PB in a dose-dependent manner and reduce antibiotic stress response confirmed by proteomics. We show that OMVs also confer protection against PB in *E.coli*, *Salmonella enterica*, and *Pseudomonas aeruginosa*. We generated artificial vesicles that validate this. OMVs were also protective against the polymyxin colistin, but not against the carbapenem meropenem or the aminoglycoside gentamicin. Finally, we show that OMVs protect *Kp* against PB in *ex vivo* and *in vivo* infection models.
In summary, our study highlights the critical role of OMVs in promoting antibiotic resistance in *Kp* and other antibiotic-resistance priority pathogens. Our data suggest that OMVs might promote bacterial replication and subsequent spreading in the host when the infection is treated with polymyxins.

Abstract No. 316

Role of myofibroblasts in lung development and repair

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The murine lung is a branched organ with a tree-like structure, where development is fulfilled postnatally. Complex cellular niches facilitate this development and multipotent stem cells play a critical role. In this context, the role of several epithelial lineages has been well established but the contribution of the mesenchymal niche remains elusive. Myofibroblasts are believed to be major players in the process of septation during postnatal alveologenesis. These cells are believed to undergo clearance upon the completion of lung development and maturation. In this work, we use state-of-the-art techniques such as lineage tracing, 3D imaging, and single-cell RNA sequencing (scRNA-seq) to characterize and spatially map these cells during the neonatal phase and trace their progeny into adulthood and aging. Our analysis uncovered the transcriptomic signature of these cells in addition to other mesenchymal populations that are also active during alveologenesis. Interestingly, we identified a population of myofibroblast-derived cells that seems to maintain a progenitor status beyond lung maturation. These findings open an avenue to target this niche to treat both neonatal and aging-related lung diseases such as bronchopulmonary dysplasia (BPD), chronic obstructive pulmonary disease (COPD) as well as acute respiratory distress syndrome (ARDS). Our work, therefore, contributes to the growing body of knowledge regarding mesenchymal complexity in the lung and the associated inter- and intracellular signaling cues that govern mechanisms of lung development and repair.

Abstract No. 317

Mobilization of the mesenchymal niche in influenza virus induced lung injury

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Viruses such as Influenza-A virus (IAV) can infect the lung and be the main cause for severe pneumonia, respiratory distress and hypoxia. IAV infection can damage both the airway and the alveolar compartment of the lung resulting in acute respiratory distress syndrome (ARDS). In the recent years, multiple studies have been published investigating how alveolar epithelium regenerates upon IAV infection. These studies gave us insights on how the epithelial cells mobilize after infection and how epithelial stem cells aid the repair of the damage inflicted by it. In this context, not much is known about mesenchymal niches and their response towards IAV infection. Taking into account that the lung mesenchyme plays a crucial role in the repair of other lung injuries such as idiopathic pulmonary fibrosis (IPF), we hypothesized that a population of mesenchymal niche is mobilized after IAV infection and plays an important role on the repair of the caused damage. In this study, we used a combination of bulk RNA sequencing, single cell RNA sequencing (scRNA-seq) and a lineage-traced approach to investigate the distribution of the mesenchymal cell populations after IAV infection. We identified a smooth muscle actin positive (ACTA2+) population of fibroblasts that responds to immune stimuli and gains regenerative and possibly anti-viral properties, as well as exert a transient fibrotic response. Further experiments will determine the cellular heterogeneity within the ACTA2+ cell population that arises in response to IAV infection as well as the mode of interaction with inflammatory and epithelial cells.

Abstract No. 318

Retrospective analysis of pulmonary gas exchange under Airway Pressure Release Ventilation in moderate or severe ARDS

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Background Since the COVID-19 pandemic ARDS and its treatment are as important as ever. Unfortunately, the last few years could not lead to new significant progress. Airway Pressure Release Ventilation (APRV) according to the Time Controlled Adaptive Ventilation (TCAV) protocol might be a promising alternative to common lung protective ventilation.
High dead space ventilation is associated with higher mortality. An estimation for dead space ventilation is the Ventilatory Ratio (VR).
Methods We retrospectively analyzed patients with moderate or severe ARDS between 2018 and 2022 in two intensive care units (ICU) who were treated with APRV compared to standard of care (SOC). Repeated measures ANOVA was used to analyze PaO_2/F_iO_2 Ratio (P/F) and VR when the ventilation mode was switched to APRV. Continuous variables were assessed using t-test, whereas categorical variables were assessed using χ^2-test.
Results In total, 107 subjects were included, 16 were switched to APRV, 7 were ventilated using APRV directly after intubation. After switching to APRV VR was significantly lower ($p=< .01$) in all subgroups. P/F Ratio was significantly higher ($p=.045$) without significant effects in separate pairwise comparison. Patients with APRV immediately after intubation had lower VR but not significantly higher P/F Ratio compared to lung protective ventilation. APRV did not reduce overall mortality compared to patients without APRV.
Conclusions APRV had lower VR in every subgroup compared to receiving SOC, implying a more effective ventilation with lower dead space ventilation. This data suggests that APRV can improve oxygenation especially if used early in patients. Due to the small sample size an improvement on overall patient outcome could not be observed.

Abstract No. 319

Role of tumor necrosis factor superfamily member 10 (TNFSF10) in SARS-CoV2-infection-associated lung damage

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Severe cases of COVID-19 are frequently associated with development of acute respiratory distress syndrome (ARDS), triggered by virus-induced hyperinflammatory response. Tumor necrosis factor superfamily member 10 (TNFSF10) is a pro-inflammatory cytokine whose mRNA expression is upregulated in COVID-19 patients and has been linked to virus-induced ARDS[1-3]. In this study, we investigate the impact of TNFSF10 on development of acute lung injury after SARS-CoV2 (CoV2) infection. We have demonstrated increased level of TNFSF10 in bronchoalveolar lavage fluid (BALF) of ICU-admitted COVID-19 patients, in supernatant of *ex-vivo* CoV2-infected human precision cut lung slices (hPCLS), primary human tissue-resident alveolar macrophages (TR-AMs) and hiPSC organoids. Treatment of human bronchial cells Calu3 and human primary alveolar epithelial cells with supernatant of *ex-vivo* CoV2-infected TR-AMs or hPCLS reduced the transepithelial electrical resistance of cell monolayers and decreased cell viability. *In vivo* infection of C57BL/*6* mice with CoV2 resulted in acute lung injury associated with significant weight loss, pulmonary edema formation and intense leukocyte infiltration of lung tissue; increased LDH activity an*d* TNFSF10 concentration in BALF. In comparison to *wt*-mice, CoV2-infected *Tnfsf10*-deficient mice did not demonstrate reduction in body weight, accumulation of protein-rich fluid in alveolar spaces and had reduced leukocytes infiltration in lungs. *Tnfsf10*-deficient mice had significantly lower mortality (0%) than *wt* animals (70%) after CoV2 infection. Together, these data indicate that TNFSF10 considerably contribute in CoV2-induced lung injury and its therapeutic targeting might reduce severity of COVID-19.

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Abstract No. 320

Bacterial extracellular vesicles repress the vascular protective factor RNase1 in human lung endothelial cells

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Background: Sepsis is one of the leading causes of death worldwide and characterized by blood stream infections associated with a dysregulated host response and endothelial cell (EC) dysfunction. Ribonuclease 1 (RNase1) acts as a protective factor of vascular homeostasis and is known to be repressed by massive and persistent inflammation, associated to the development of vascular pathologies. Bacterial extracellular vesicles (bEVs) are released upon infection and may interact with ECs to mediate EC barrier dysfunction. Here, we investigated the impact of bEVs of sepsis-related pathogens on human EC RNase1 regulation.
Results: bEVs from the sepsis-associated bacteria *Escherichia coli*, *Klebsiella pneumoniae* and *Salmonella enterica* serovar Typhimurium were isolated via ultrafiltration and size exclusion chromatography and used for stimulation of human lung microvascular ECs. Thereby, gram-negative bEVs from *E.coli*, *Klebsiella* and *Salmonella* significantly reduced RNase1 mRNA and protein expression and activated ECs, while TLR2-inducing bEVs from *Streptococcus pneumoniae* did not. These effects were mediated via LPS-dependent TLR4 signaling cascades as they could be blocked by Polymyxin B. Additionally, LPS-free *ClearColi*™ had no impact on RNase1. Further characterization of TLR4 downstream pathways involving NF-κB and p38, as well as JAK1/STAT1 signaling, revealed that RNase1 mRNA regulation is mediated via a p38-dependent mechanism.
Conclusion: Blood stream bEVs from gram-negative, sepsis-associated bacteria reduce the vascular protective factor RNase1, opening new avenues for therapeutical intervention of EC dysfunction via promotion of RNase1 integrity.

Abstract No. 321

Human Alveolospheres to characterize AT2 cell immunomodulation and regeneration potential upon *Chlamydia pneumoniae* infection.

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AT2 cells play an important role in homeostasis and regenerative challenges of the lung alveoli to keep them functional. Stem cell potential enables these cells to self-renew, but these cells perform immunomodulatory tasks in addition to regenerative ones. They constitutively express MHC-II molecules and function as non-professional antigen-presenting cells. Specific ablation of MHC-II on these cells in a Cre-lox mouse model resulted in an impaired antiviral immune response, suggesting an essential role in the robust and potent defence against respiratory pathogens. 75% of the population comes into contact with the intracellular bacterium *Chlamydia pneumoniae* by the age of 65. AT2 cells, comparable to viral diseases, represent a target cell whose infection leads to intracellular proliferation. Chlamydiae use this intracellular replication for immune evasion and rely on the host cell's metabolism. In response to host defence mechanisms, persistent forms with low metabolic activity are formed, which can survive intracellularly. Under transient or even chronic immunosuppression, this represents possible reactivation foci. *Ch. pneumoniae* has been associated with several inflammatory conditions of the respiratory tract and other organ systems. We found that infection changes the regenerative potential of the AT2 cells while the cells de-differentiate. Moreover, immunomodulatory receptors as well as cytokine responses are being modulated. We aim to characterize this process and decipher chlamydial infection-specific changes to guide the search for new therapeutic approaches for chronic-inflammatory conditions and acute atypical pneumonia.

Abstract No. 322

The rare villin-expressing cell of the murine lower airway is a neuroendocrine rather than a tuft cell

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Previous studies reported an enigmatic rare cell in the mouse tracheal epithelium displaying an apical tuft of villin-immunoreactive microvilli. This study aimed to clarify the identity of this ill-defined cell type.
Wild-type and genetically modified mice, including *Pou2f3*$^{-/-}$, *Chat*-eGFP, *Vil1-Cre/ROSA*$^{mT/mG}$ and *Avil-Cre/ROSA*$^{mT/mG}$ mice, were used to assess villin expression in airway epithelium, using electron microscopy, immunohistochemistry and *in silico*-analysis of publicly available sequencing data. Electron microscopy revealed cells with neuroendocrine cell features (i.e. large dense core vesicles) as the only rare microvillous epithelial cell type in the trachea of mice lacking tuft cells (*Pou2f3*$^{-/-}$). Immunolabeling revealed extensive co-labeling of villin and neuroendocrine-specific-cell markers in wild-type tracheas (64% of PGP9.5^{+} cells), but only minimally in lungs (≈1% of CGRP^{+} cells). *Vil1-Cre* activity was detected in 22% of tracheal PGP9.5^{+} and 47% CGRP^{+} bronchopulmonary neuroendocrine cells. Tuft cells, in contrast, were only rarely villin^{+} (2% of GNAT3^{+} cells), but advillin^{+} (85% of *Chat*-eGFP^{+} cells). Accordingly, *Avil*-eGFP was detected in 75% of TRPM5^{+} tuft cells, but not in neuroendocrine cells. *In silico*-analysis of publicly available sequencing data of murine tracheal epithelial cells revealed *Vil1*-mRNA expression in neuroendocrine cells while *Avil*-mRNA is expressed in tuft cells.
The present study identifies the enigmatic villin-expressing cells in the lower airways as a cell population hidden amongst the neuroendocrine cells. Thus, advillin, rather than villin, is a tuft cell marker in the airways, which shall be considered in interpreting findings based on the use of villin to identify tuft cells, which has been common practice in the past.

Abstract No. 323

K_{Ca} channels as amplifiers of TRPV4-mediated pulmonary edema formation

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Mechanical ventilation is life-saving in patients with acute respiratory failure, but can also inflict ventilator-induced lung injury (VILI) and exacerbate endothelial barrier failure. The mechanosensitive transient receptor potential vanilloid 4 (TRPV4) channel mediates Ca^{2+} influx and can activate Ca^{2+}-activated K^+ (K_{Ca}) channels, which may in turn increase the electrochemical gradient for Ca^{2+} influx. As such, K_{Ca} channels may promote stretch-induced barrier failure and aggravate VILI.
Male mice were ventilated for 2 h with low or high tidal volumes in the presence or absence of K_{Ca} antagonists (apamin, charybdotoxin, TRAM34). Changes in endothelial Ca^{2+} concentration ($[Ca^{2+}]_i$) were quantified by ratiometric imaging of isolated-perfused mouse lungs subjected to CPAP elevation, or in human pulmonary microvascular endothelial cells (HPMECs) in response to TRPV4 activation with or without inhibition of K_{Ca} channels. Analogously, changes in endothelial K^+ concentration ($[K^+]_i$) and membrane potential were imaged *in vitro*.
Inhibition of K_{Ca} channels, specifically IK1 attenuated characteristic features of lung injury *in vivo*. Accordingly, inhibition of IK1 channels in isolated lungs reduced the sustained $[Ca^{2+}]_i$ response to CPAP elevation. In HPMECs, TRPV4-mediated Ca^{2+} influx decreased $[K^+]_i$, causing membrane hyperpolarization. This response was prevented by inhibition of K_{Ca} channels, whereby inhibition of IK1 channels showed the most pronounced effect. Inhibition of K_{Ca} channel downstream of TRPV4 reduced endothelial $[Ca^{2+}]_i$ *in vitro*.
K_{Ca} channels, specifically IK1, act as amplifiers of TRPV4-mediated Ca^{2+} influx in the lung endothelium and can establish a feed-forward cycle that drives endothelial barrier disruption and promotes the progression of VILI.

Abstract No. 324

Epithelial Changes During Acute Injury in Precision-Cut Lung Slices

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Precision-cut lung slices PCLS represent a model of acute lung injury for the mature AT2 cells. We hypothesize that this model could be useful for the identification of lineage-traced cells involved in the repair process.
PCLS were obtained from human Donor lungs, Tom^{Sftpc} (SftpcCreERT2/+; tdTomatoflox/flox) and Gfp^{Sftpc} (Gfp is directly under the control of the *Sftpc* promoter) mouse lungs were collected at different timepoints to evaluate dynamic changes during lung tissue culture by qPCR. For live imaging of transgenic lung mice, PCLS were maintained in a heated and humidified chamber during imaging.
qPCR analysis of human Donor PCLS over time indicated that the expression of EPCAM and SCGB1A1 did not display significant changes while the expression of *SFTPC* decreased dramatically. We also found increased expression of FGF10 over time suggesting the presence of a repair response in the human PCLS. We also performed long-term live imaging of PCLS in Tom^{Sftpc} and Gfp^{Sftpc} mice and followed the lineage-positive cells for 8 days. Analysis and quantification of the IF images showed a significant presence of Tom^{Sftpc} near the bronchial region, while a decrease was observed in the alveolar region. Interestingly, this phenomenon was not observed with the Gfp^{Sftpc} PCLS indicating that the lineage-traced cells in and around the bronchi at later time points do not express Sftpc. Despite the presence of agarose in the airspace of the PCLS, significant migration of Tom^{Sftpc} was observed.
Lineage tracing of Sftpc+ cells in mouse PCLS allowed the visualization of an unexpected population of progenitors which are currently analyzed.

Abstract No. 325

S100A9 is indispensable for survival of pneumococcal pneumonia in mice

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S100A8/A9 has important immunomodulatory functions in antibacterial immunity, but its relevance in focal pneumococcal pneumonia is poorly defined. Here we examined the role of S100A9 in pneumococcal pneumonia. We found that S100A9 was significantly increased in BAL fluids of patients with bacterial pneumonia and correlated positively with PCT, CRP and SOFA scores. Opposed to WT mice, *S. pneumoniae*-infected S100A9 KO mice exhibited significantly elevated Zn^{2+} levels in lungs, which led to drastically increased bacterial loads together with substantially reduced survival, along with massive release of neutrophil elastase, which resulted in degradation of opsonophagocytically important collectins surfactant proteins A and D. This inflammatory phenotype was completely corrected in *S. pneumoniae*-challenged, irradiated S100A9 KO mice upon WT bone marrow transplantation. Heterodimeric S100A8/A9 protein inhibited pneumococcal outgrowth *in vitro* by chelating divalent metal ions magnesium, zinc and manganese. Accordingly, therapy of *S. pneumoniae*-infected S100A9 KO mice with S100A8/A9 protein led to significantly decreased lung bacterial loads and lung injury. Collectively, S100A9 regulates essential antibacterial immune mechanisms of the lung by controlling both levels of divalent cations and antibacterial effector proteins SP-A and -D in pneumococcal pneumonia in mice. Moreover, S100A9 appears to be a promising biomarker to distinguish patients with bacterial from those with viral pneumonia.

Abstract No. 326

Causes and risk factors for absenteeism among medical staff in German specialized lung clinics during the COVID pandemic

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Background: Staff shortages pose a major challenge to the health system. To clarify the role of different causative factors we investigated staff absenteeism during the COVID pandemic.
Methods: A study-specific online questionnaire was designed to assess the private and professional impact of the pandemic on healthcare workers (HCW) from specialized lung clinics. Staff members at 7 clinics across Germany were surveyed from 1 December 2021 to 23 December 2021. The current analysis addresses pandemic-related absenteeism.
Results: 1134 HCW (55% female) participated. 72.8% had received at least one vaccination dose at the time of the survey, and 9.4% reported COVID-19. Of those with positive tests, 98% reported home quarantine for median (IQR) 14 (12-17) days; 10.3% of those who ultimately tested negative also reported quarantine periods of 14 (7-14) days. 32.2% of vaccinated respondents reported absenteeism due to vaccine reactions of 2 (1-3) days. Overall, 37% (n=420) of HCW reported pandemic-related absenteeism, with 3524 total days of absenteeism, of which 2828 were due to illness/quarantine and 696 to vaccination effects. Independent risk factors for pandemic-related absenteeism ≥5 days included COVID-19 (OR 367.4, $p<0.001$), effects of vaccination (OR 2.5, $p<0.001$), and concerns related to long-term effects of COVID-19 (OR 1.3, $p<0.001$) or vaccination (OR 1.2, $p=0.043$).
Conclusion: Staff shortages due to quarantine or infections and vaccine reactogenicity have put a strain on German respiratory specialists. The fact that staff concerns also contributed to absenteeism may be helpful in managing future pandemic events to minimize staff absenteeism.

Abstract No. 327

Interfering with different structural components of the ribosome and ribosomal biogenesis may alter influenza A virus infection in lung epithelial cells

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Background: Influenza A virus (IAV) infections cause seasonal endemics and sporadic pandemics with high death tolls. Severe disease, such as virus-induced pneumonia and ARDS, is accompanied by alterations of global protein synthesis and the host translational apparatus. However, viral proteins are selectively translated under these conditions. Although several elements of the mechanism of IAV-induced *shutoff* have been revealed recently, little is known about the role of the ribosome and the ribosomal proteins (RPs) in this context. Since there is increasing evidence that RPs have important functions in pathological processes such as cancer and viral infections, we investigated the potential role of RPs and ribosomal biogenesis upon IAV infection.
Methods/Results: First, we identified potentially involved components of the ribosome *in vivo* by utilizing RNAseq in alveolar epithelial cells of infected mice. Next, we confirmed our findings *in vitro* by transcriptomic and proteomic analyses of A549 and MLE-12 cells after IAV infection. These data show significant changes of the RPs *Rps24*, *Rpl5, Rpl10 and Rpl11* as well as the regulatory protein *Rrs1* at mRNA levels. Depleting these factors by RNAi had contrary impact on the course of infection: downregulation of *Rps24* or *Rpl10* lead to much stronger infection as measured by the amount of viral RNA and proteins, whereas downregulation of *Rpl5, Rpl11 and Rrs1* inhibited viral propagation.
Conclusions: Our results suggest a central involvement of ribosomal components upon IAV infection. Further research focusing on the underlying mechanisms (e.g. ribosomal stress pathways) will enable us to characterize the “pro-viral ribosomal landscape” and may lead to selective therapeutic approaches against respiratory failure upon IAV infection.

Abstract No. 328

Self-organising-map based clusters identify patients at risk for long-term mortality in community-acquired pneumonia in CAPNETZ

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Rationale: Community-acquired pneumonia (CAP) remains a disease with high morbidity and mortality. While tools and scores to predict short-term prognosis of CAP, *e.g.* the CRB65-Score, have been developed, there is urgent need for early identification of patients at risk for adverse mid- or long-term outcome. Here, we report cluster analysis of CAP-patients clinical attributes at hospital admission with subsequent curation of an attribute panel predicting adverse clinical 31-to-180-day outcome.
Methods: Data from the German prospective CAPNETZ cohort was extracted and clinical attributes of patients (n=7,248) at hospital admission clustered by means of self-organising-maps (SOM). Differential clinical attributes of clusters identified by outcome-based clustering were identified and used for attribute-based clustering. Clusters identified by attribute-based clustering were analysed for further differences in clinical attributes.
Results: SOM-clustering identified a panel of eleven easily accessible clinical attributes to predict 30-day as well as 31-to-180-day mortality in 7,248 CAP patients. Clustering based on these attributes yielded 15 clusters, identifying differential clinical phenotypes with high risk of mortality in the 31-to-180-day timeframe, the 30-day timeframe, both periods, or very low overall mortality, respectively.
Conclusions: We found that (i) SOM-based clustering analysis of clinical attributes in CAP is both possible and generating information of clinical value, (ii) eleven routine clinical attributes can be used to predict short- or mid-term mortality in CAP, (iii) patients at low or high-risk bear differential clinical phenotypes which are easily recognizable and inform further treatment-intervention trials.

Abstract No. 329

Expression of Transient Receptor Potential (TRP) channels in murine lung Insterstial Macrophages (IM)

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Objective: Interstitial macrophages (IM) are essential for organ homeostasis, immune-regulation and an autonomous immune response. These functions are achieved through macrophage polarization characterized by pro-inflammatory M1 and pro-fibrotic M2 states. Transient receptor potential (TRP) channels, non-selectively permeable to Ca 2+ ions, are functionally expressed in various immune cells [1]. Single cell transcriptomic studies have revealed the heterogeneous nature of the IM in steady state [2]. This immense diversity combined with the remote parenchymal localization and low counts are limiting factors for isolation of IM for further in vitro studies.
Methods: IM were isolated by fluorescence-activated cell sorting (FACS) for specific surface markers [2] or propagated in co-culture with lung fibroblasts after enzymatic digestion of the lungs [3]. Polarization of IM to M1 and M2 cells was performed by application of lipopolysaccharide (LPS) and interferon γ (IFNγ) or interleukin 4 (IL-4), respectively. MRNA expression of TRP channels and cell markers was quantified by reverse transcription quantitative (RT-q) PCR.
Results and Conclusion: In this study, we were able to isolate and culture IM from wild-type (WT) and TRP-deficient mouse models and explore expression of TRP channels in naive and stimulated M1 and M2 states. TRPC1, TRPC6, TRPM2, TRPM7, TRPV2, TRPV3, and TRPV4 were predominantly expressed in naïve IM. TRPM2, TRPV4, TRPV2 were up-regulated in the M1 state, while there was no difference in M2 cells. In the future, comparing WT and TRP-deficient IM will reveal important TRP functions in this cell type.

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Contribution of a Disease-spanning Working Group: Lung Environment Interaction

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Abstract No. 330

Alveolar micromechanics in TGF-β1 overexpressing mice

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Although being a life-saving method, mechanical ventilation can aggravate lung injuries. In an injured lung, inflammation and surfactant dysfunction leads to heterogeneous ventilation and abnormal alveolar micromechanics. Due to the loss of stability, small regions of collapse (microatelectases) appear. Repetitive opening and closing can damage the blood-gas barrier (atelectrauma) while open alveoli get overstretched (volutrauma). Hence, recruitment and overstretch should be balanced in injured lungs during mechanical ventilation. In my study I investigate the degree of alveolar collapse and stretch as a function of airway opening pressure after adenoviral transfer of TGf-ß1, which is known to result initially in surfactant dysfunction. One week after gene transfer, lung mechanics were measured during ventilation at positive end expiratory pressures (PEEPs) between 12 and 0 cmH_2O. Afterwards, lungs were fixed by vascular perfusion at different airway opening pressures (Pao) and subjected to design-based stereology. Lung mechanics such as tissue elastance (H), tissue dampening (G) and compliance (Cst) were significantly worse in the AdTGF-β1 group over the range of PEEP-levels. Minimal tissue elastance was observed between PEEP 8 and 5 cmH_2O; higher and lower PEEP values showed significantly stiffer lungs. The Stereology of lungs fixed at a Pao of 20, 10, 5 and 2 showed progressive alveolar derecruitment in AdTGF-β1 mice but not in controls. In the AdTGF-β1 group, the amount of collapsed septa as well as alveolar surface and numbers of alveoli was closest to healthy controls at a fixation pressure of 10 cmH_2O. Together, the data suggest an optimal balance of derecruitment and stretch at a PEEP of 8-10.

Abstract No. 331

Impact of GM-CSF on lung epithelial cell injury and repair in severe influenza virus pneumonia

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GM-CSF (granulocyte-macrophage colony-stimulating factor) is a well-known myeloid growth factor that has been implicated in alveolar epithelial type 2 cell (AECII) repair after lung damage. However, the distinct GM-CSF-mediated signaling pathways driving epithelial (stem) cell repair during viral infections remain elusive. Therefore, this study aimed to identify the molecular mechanisms involved in the epithelial regenerative effects of GM-CSF following influenza A virus (IAV)-induced injury.
During IAV infection, GM-CSF is predominantly expressed by BASC and AECII which significantly induces their proliferation. Furthermore, in our *in vitro* bronchioalveolar lung organoid (BALO) model the GM-CSF/GM-CSF receptor axis was necessary for proper alveolarization. Bulk RNA sequencing revealed an upregulation of catabolic pathways in BASC of infected csf2-/- compared to infected WT mice. The treatment of infected AEC from csf2-/- mice with recombinant GM-CSF caused downregulation of AMPK, a master regulator of metabolic pathways, and activation of mTORC1. Accordingly, cell cycle genes, *Ccnd1* and *cMyc*, were upregulated in infected WT but not in csf2-/- AECII. Notably, treatment of lung organoids with AMPK inhibitor or mTORC1 activator supported organoid forming efficiency and alveolarization. Lastly, IAV-infected csf2-/- mice treated with the AMPK inhibitor Compound C displayed a significant increase in BASC and AECII proliferation and promoted epithelial barrier repair.
In summary, these data indicate that the protective effects of GM-CSF in BASC and AECII after viral lung injury are mediated by downregulation of AMPK signaling which induces mTORC1 activation, therefore, supporting epithelial stem cells´ proliferation and restoration of the alveolar barrier function.

Abstract No. 332

TRPM2 channels in oxidative stress-induced pulmonary endothelial barrier dysfunction

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RATIONALE The barrier formed by the microvascular pulmonary endothelium (MPE) is essential for gas transport and host defense, and its disruption can result in edema formation and respiratory failure. The Transient Receptor Potential M2 (TRPM2) protein is a mediator of oxidative stress-induced vascular permeability. Activation of TRPM2 through oxidative stress causes an influx of Ca^{2+}, triggering internalization and degradation of junctional proteins. However, recent reports suggest that a disintegrin and metalloproteinase domain-containing protein 10 (ADAM10), which is responsible for ectodomain shedding, is also activated by TRP-medated Ca^{2+} influx[1]. Here, we sought to determine whether ADAM10 was involved in TRPM2-mediated MPE barrier dysfunction.

METHODS We applied a model of H_2O_2-induced oxidative stress to primary human MPE cells. Barrier response was quantified using electric cell-substrate impedance sensing, and changes in the expression of junction proteins were assessed with Western blot. The effects of H_2O_2 exposure were investigated under conditions of TRPM2 silencing through siRNA-mediated knockdown and the inhibition of ADAM10.

RESULTS H_2O_2 rapidly reduced MPE barrier resistance, which was matched by a dramatic reduction in VE-Cadherin expression and the formation of a 35kDa VE-Cadherin degradation product. Interestingly, this apparent degradation of VE-Cadherin was rescued by both TRPM2 knockdown and by inhibition of ADAM10.

CONCLUSIONS Our results suggest that TRPM2 mediates oxidative stress-induced barrier dysfunction in the MPE, and that the established pathway by which TRPM2 activation leads to VE-Cadherin degradation should be amended to include ectodomain shedding through the activation of ADAM10.

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Abstract No. 333

Angiotensin 1-7 and ACE2-overexpression results in decreased IL-1ß levels following NLRP3 inflammasome stimulation

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Background: COVID-19 is a highly inflammatory disease which can lead to ARDS. Angiotensin converting enzyme 2 (ACE-2) enables entry of Sars-CoV2 and converts angiotensin (Ang) II into Ang(1-7). Ang(1-7) counters inflammatory effects of the Ang-II-AT1R axis. We aimed to analyze Ang(1-7) BAL fluid levels in ARDS and the potential of ACE-2 overexpression (OE) and Ang(1-7) to inhibit inflammasome activation in vitro.

Methods: HEK-293T-Cells and air-liquid-interface (ALI) cultures together with U937 monocytes were cultured and stimulated with Ang-II, Ang(1-7) and A779 (MasR inhibitor). Inflammasome activation was tested via LPS and nigericin. ACE2 OE in HEK293T cells and ALI-cultures was achieved via lentiviral vector transduction. IL-1ß and Ang(1-7) levels were measured in culture supernatants and BAL fluid from ARDS patients.

Results: Ang(1-7) was markedly reduced in BAL fluid in COVID-19 and other viral / bacterial ARDS compared to healthy controls. Lower Ang(1-7) BAL fluid levels were associated with mortality ($p=0.018$). In cell-cultures, HEK293T and ALI co-cultures (with U937-cells) inflammasome activation could be induced. ACE2 OE led to a reduction in IL-1ß levels in both HEK293T ($p<0.001$) and ALI co-cultures ($p=0.0165$). Ang(1-7) markedly reduced IL-1ß levels in HEK293T ($p<0.001$) and ALI co-cultures ($p=0.01$) regardless of ACE-2 OE. ACE2 OE however increased the production of Ang(1-7). Inhibition of MasR (Ang-1-7-receptor) reversed these effects.

Conclusions: IL-1ß due to inflammasome activation can be inhibited via Ang(1-7). Production of Ang(1-7) was increased by ACE-2 OE. MasR-inhibition completely reversed the protective effect of Ang(1-7). Lower Ang(1-7) levels in ARDS were associated with mortality. MasR agonists may be beneficial in some ARDS patients.

Abstract No. 334

Increased collagens and matrisome-specific antibodies in bronchoalveolar space define COVID-19 patients from other types of pneumonia

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Infectious respiratory diseases hold the rank of the fourth most fatal group of diseases worldwide. Despite multiple emerging COVID-19 studies, most of them lack suitable comparisons to other types of pneumonia with similar severity levels. In this study, we utilized the SCRIPT cohort at NWU Chicago to conduct longitudinal profiling of bronchoalveolar lavage fluid (BALF) and matched plasma samples to discern pathogen-specific differences during disease progression. To achieve this, we used high-resolution mass spectrometry to analyze BALF and matched plasma samples from COVID-19 (n=14), bacterial pneumonia (n=8), influenza (n=8) patients, and non-pneumonia controls (n=8) at four-time points after intubation in the intensive care unit.
Our analysis of COVID-19 patients' BALF revealed specific enrichments in immunoglobulins, blood clotting proteins, and collagens, suggesting an increase in fibrogenesis and B-cell immunity already at the time of intubation compared to other bacterial or viral types of pneumonia. Furthermore, we utilized our recently developed Differential Antigen Capture assay to examine the binding specificities of plasma antibodies in all patients, identifying potential cross-reactivity with lung matrisome proteins in COVID-19 cases. In contrast, bacterial pneumonia was rich in neutrophil degranulation proteins, while influenza samples contained high levels of mucins. Notably, the differential signatures observed in BALF were partially conserved in patients' plasma. In summary, we identified specific proteomic signatures of COVID-19 compared to influenza and bacteria-driven pneumonia, suggesting increased early onset local B-cell immunity and fibrogenesis in COVID-19.

Abstract No. 335

Role of repair-supportive mesenchymal cells (RSMCs) in airway epithelial regeneration

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Stem and progenitor cells, as well as the surrounding niche, function in a coordinated and dynamic manner to promote tissue regeneration. The crosstalk between these cells within the lung tissue is often highly complex. Recent efforts have helped simplify such complexity, especially through the use of genetic lineage tracing. We have identified a novel cell population that we termed “repair-supportive mesenchymal cells” (RSMC), which is distinct from airway smooth muscle cells (ASMC) and is critical for regenerating the conducting airway epithelium by secreting fibroblast growth factor 10 (FGF10). We recently showed that RSMCs at least in part derive from glioma-associated oncogene 1 (*Gli1*)-expressing mesenchymal cells. In this project, we plan to delineate the complexity of the RSMC lineage and assess the contribution of other known mesenchymal subsets to this lineage. Our approach includes lineage tracing, single-cell RNA sequencing (scRNA-seq), and lung organoids. We will also focus on cell-cell communication networks between airway progenitor/stem cells and RSMCs. The attained data might potentially shape novel treatment strategies to treat airway diseases.

Abstract No. 336

Responses of human microvascular lung endothelial and bronchial epithelial cells to serum with divergent profiles and magnitudes of inflammatory markers

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The hyper-activation of the immune system with cytokinemia is associated with more severe lung endothelial (EC) and epithelial cell (EpC) damage. It remains unclear which specific profiles and levels of pro- and anti-inflammatory markers can induce lung cell activation/damage. We focused on the relationship between shapes and magnitudes of serum inflammatory markers in COVID-19 patients, and how these latter affect human microvascular lung EC and bronchial EpC, *in vitro*. Based on the cytokine/chemokine multiplex assay, we found significantly higher levels of soluble ICAM-1 and IL-17 in severe (n=10) than in moderate (n=9) COVID-19 patients. Next, serum from each patient was added to EC (5%) and EpC (2%) for 18 h. We observed a marked induction of IL-8/CXCL8 release and expression in EpC and EC exposed to the serum of severe but not moderate COVID-19 patients. We also analyzed cell responses to patient serum based on the levels of inflammatory markers, independently of the disease severity. Serum containing higher levels of chemokines (IL-8 and MCP-1), and soluble ICAM-1 significantly activated both lung EC and EpC. Specifically, serum containing higher levels of soluble ICAM-1 increased FITM2 mRNA in EpC and VEGFA mRNA in EC. The EC treated with serum containing less MCP-1 and IL-8 showed a higher expression of PECAM1. We demonstrate divergent responses of EC and EpC to patient serum containing different levels and profiles of inflammation markers and that *in vitro* models of human lung cells can be useful to study respiratory system injuries.

Abstract No. 337

Preserved prenatal lung growth in fetal MRI with mild omicron infection during pregnancy

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Previous studies have shown a reduction in fetal lung volume in the offspring of women with mild COVID-19 symptoms during the pre-vaccination era. As the virus has mutated rapidly, it is crucial to investigate the impact of the latest SARS-CoV-2 omicron variant.
Fetal lung volumes in 24 women with omicron variant infection during pregnancy were compared to non-COVID control groups. A multivariate analysis was conducted to also compare the results with additional MRI data from pregnant women who had a mild infection with the SARS-CoV-2 alpha variant. Postnatal follow-up was conducted in ten infants with SARS-CoV-2 omicron exposure during pregnancy.
In omicron-exposed fetuses, lung volume did not differ significantly from non-COVID control group (p=0.76). Multivariate analysis revealed a significant association of virus-induced effects on fetal lung volume (omicron vs. alpha, p=0.04). At birth follow-up in omicron-exposed infants exposure during pregnancy revealed adequate birth weight and no acute postnatal respiratory distress.
The tropism of the omicron variant, which has been observed to replicate less efficiently in the lung parenchyma but more prominently in the upper airway epithelium, likely reflects its biological properties. In our recent study, we investigated the impact of this variant on fetal lung development and found no significant effect on fetal lung volume. This is in contrast to our earlier research, which demonstrated a reduction in fetal lung volume in the offspring of women with mild COVID-19 symptoms caused by the alpha variant during the pre-vaccination era.
Demonstrating the importance of MRI in detecting prenatal lung injury, our results further reflect on the ameliorating impact of vaccination.

Abstract No. 338

LOX-1: Differentiation of an immune-relevant receptor in human and mouse

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Introduction: Lectin-like oxidised low-density lipoprotein receptor-1 (LOX-1) is known as a major receptor for oxidised low-density lipoprotein (oxLDL) and plays a significant role in atherosclerosis, but recent research has shown its involvement in cancer, ischemic stroke, and diabetes. As a pattern-recognition receptor (PRR), LOX-1 is involved in the activation of cells and inflammatory processes. Therefore, it is plausible that LOX-1 may be involved in infections or the host's response to them. However, further research is required to fully understand the extent of LOX-1's involvement in this area.
The objective was to conduct a literature search to gather information on all interactions between LOX-1 and pathogens, as well as the host response to infections triggered by LOX-1.
Results: We conducted a literature search that demonstrated the involvement of LOX-1 in several infections. We observed potential differences between human and murine LOX-1 and their corresponding signaling pathways. Based on this, we analyzed structural differences genomically and at the protein level and compared these with data from other PRRs. The results reveal both similar and different structures in human and murine LOX-1 and provide clues to the possible origins of the different modes of interaction. Our findings indicate that mouse models may not be entirely suitable for studying LOX-1, and thus, caution should be exercised when using them. Instead, human cell models should be preferred for investigating the role of LOX-1 in host responses to infections. Future research should aim to better understand the interaction and binding of LOX-1 with various human pathogens to enhance our understanding of the role of LOX-1 in the infection process and identify potential pathways of inhibition.

Abstract No. 339

Blood eosinophil count as predictor of disease severity in hospitalized patients with community-acquired pneumonia

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Background and objective: Mortality in patients with community-acquired pneumonia (CAP) continues to be high. Predictors of disease severity are required to improve patient management. This study investigated blood eosinophil levels at hospital admission as independent biomarker for predicting severity of CAP.

Methods: We retrospectively reviewed ≥18-year-old patient cases hospitalized with CAP from 2009 to 2020 using data from electronic health records of five German university hospitals from the Medical Informatics in Research and Care in University Medicine (MIRACUM) consortium. Patients were divided into an eosinopenia group (eosinophils ≤50/µL) and non-eosinopenia group (eosinophils >50/µL). Risk of death and mechanical ventilation were analyzed in a multivariate model. Length of mechanical ventilation, length of stay in survivors, and time to in-hospital death were calculated.

Results:
Overall, 6748 patient cases were included in the analysis. Of those, 4060 cases had blood

eosinophils ≤50/µL, while 2688 cases had eosinophils >50/µL. In the eosinopenia group, in-hospital mortality (14%; $p=1.272 \times 10^{-06}$) and risk of mechanical ventilation (19%; $p=7.594 \times 10^{-06}$) were increased compared to the non-eosinopenia group (9 and 14%, respectively). Length of stay in survivors in the eosinopenia group was longer (8.41 days; $p=1.380 \times 10^{-05}$) versus the non-eosinopenia group (7.64 days), while length of mechanical ventilation did not differ (p=0.152) between groups. Time to in-hospital death was shorter in the eosinopenia (6.73 days; p=0.001) versus the non-eosinopenia group (8.92 days).

Conclusions: Eosinopenia ≤50/µL may be used as independent predictor of disease severity in CAP. Future prospective studies are required to confirm our results.

Abstract No. 340

Structural and functional alterations of the mechanically ventilated pre-injured lung

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The COVID-19 pandemic revealed that mechanical ventilation is an understudied field with opportunities for preventing ventilator-induced lung injury (VILI). Our research centers on the structural alterations of the lung and aims to generate new data about the functional implications of mechanical ventilation of pre-injured lungs. To investigate the hypothesis that microatelectases serve as “stress concentrators” and lead to ever-increasing heterogeneity in the ventilated lung we use an animal model to gain mechanical, structural, and biochemical data. Rats are instilled with bleomycin and ventilated 24 hours later for varying durations (2h, 4h, and 6h, n = 15 per group) with a positive end expiratory pressure (PEEP) = 1 cmH2O and tidal volume of 10 ml/kg body weight. Additionally, another group with a more protective mode of ventilation (PEEP = 5 cmH2O) is used to test if alveolar stabilization reduces injury. Repetitive measurements of lung mechanics during ventilation track injury progression at the organ scale, design-based stereology provides structural quantitative data and molecular changes in the tissue and BAL are analyzed biochemically. Combining and correlating these data, we are able to evaluate the impact on lung function. Our data shows that the low PEEP ventilation leads to increased alveolar derecruitment, especially after more than four hours of ventilation. A decrease in inspiratory capacity, alveolar surface area, and number of open alveoli suggests progressive derecruitment at the alveolar level. Additionally, malfunction of the surfactant protein B system and an increased probability of microatelectases in close proximity to regions of collapse, provides us with convincing evidence that stress concentrations are important in VILI pathogenesis.

DA Diffuse Parenchymal Lung Disease: Abstract No. 401 – 457

Abstract No. 401

Considering the eosinophil as a mediator of disordered lung development in bronchopulmonary dysplasia

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Bronchopulmonary dysplasia (BPD) is the most common chronic pulmonary complication of preterm birth. Survivors of BPD have chronic respiratory symptoms and abnormal lung function into adulthood, making BPD a leading cause of pediatric lung disease. Premature infants with BPD have increased eosinophils and eosinophil granule proteins in venous blood and tracheal aspirates of affected infants, and were positively correlated with duration of oxygen supplementation in affected infants. Collectively, these data suggest eosinophils may be pathogenic mediators of disordered lung development in affected infants.

Our objectives are three-fold: (i) assessing the physiological role of eosinophils in lung development, (ii) understanding if eosinophils contribute to BPD and if so, how are they recruited. Finally, (iii) is the presence of eosinophils sufficient to trigger BPD. Objectives will be achieved through: pharmacological eosinophil depletion, genetically driven eosinophil depletion, genetic depletion and overexpression of IL-5.
Early data indicates that blood and lung eosinophil levels change dynamically over the course of early lung development (first 14 days of post-natal life) in mice. Newborn pup exposure to supraphysiological levels of inspired oxygen significantly increased eosinophils in lungs of affected pups. Compared to lungs of pups treated with a pharmacological intervention, levels of eosinophils in the lung were greatly depleted in both normoxia and hyperoxia.
Our preliminary data reveal hyperoxic injury to developing mouse lungs result in pronounced recruitment of eosinophils to the developing lung. Reversely, pronounced recruitment of eosinophils to the developing mouse lung can be depleted with pharmacological intervention targeting eosinophil recruitment.

Abstract No. 402

Ex vivo testing of the anti-fibrotic efficacy of drugs on idiopathic pulmonary fibrosis by means of long-term culture of human precision-cut lung slices

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The short-term culture of human precision lung slices (PCLS) is a well-established method for drug testing. However, cell viability is maintained for only up to five days. To test the efficacy of anti-fibrotic drugs and accurately predict fibrosis modulation in patients, longer culture periods and the use of patient tissue are needed. In this study, we developed a method to culture PCLS from IPF lung tissue for more than four weeks while maintaining cell viability and tested several established drugs.
To culture PCLS, freshly obtained human IPF lung explants were filled with agarose and sliced into approximately 300 µm-thick slices using a vibrating microtome. The PCLS were then washed and plated onto 24-well plates and treated with various anti-fibrotic drugs. Culturing lasted for six weeks, and media exchange was performed every other day.
Under our conditions, the cultured PCLS remained viable for up to six weeks. We observed a reduction in the alveolar space and shrinkage of the PCLS area in IPF lung tissue compared to the starting slices (day 0), while healthy lung tissue did not shrink. Masson-Goldner staining showed an increase in fibrosis areas and fresh collagen fiber formation over time. Additionally, de novo formation of organoid-like structures was observed. Furthermore, we determined the CTGF and TGF-β concentrations in the culture supernatant by ELISA, which both increased in parallel with the increasing remodeling.
Our novel PCLS long-term culture of IPF tissue allows for meaningful ex vivo testing of the antifibrotic effects of drugs for the first time. Interestingly, fibrosis and tissue remodeling in IPF tissue continue to progress during culture.

Abstract No. 403

Exploring Changes in Cell-Cell Communication Pathways in Lung Fibrosis: A Spatiotemporal Analysis Approach

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Understanding the molecular mechanisms of fibroblasts to myofibroblasts transdifferentiation as a key pathomechanism of lung fibrosis may lead to the development of new therapeutic strategies for treating this fatal disease. Factors secreted by immune cells drive myofibroblast transdifferentiation and persistence. In contrast to conventional pathways that activate myofibroblasts (eg. TGFβ1), additional immunosecreted factors may exist that drive fibrogenesis. This study aimed to identify novel immunosecreted profibrotic factors that drive intercellular communication between immune cells and (myo)fibroblasts. First, we performed a meta-analysis of pre-existing single-cell RNA data from a human ex vivo fibrosis model and an integrated IPF cell atlas. For validation, we used human cytokine arrays evaluating the supernatants of human precision-cut lung slices (hPCLS) treated with a fibrotic cocktail and a novel antifibrotic compound series (N23Ps). This analysis led to the identification of specific ligands that might drive or block myofibroblast formation. Selected ligands were tested in a medium-throughput fibroblast-to-myofibroblast transdifferentiation assay using IPF-patient-derived primary human fibroblasts. The results showed a distinctive set of cytokines deregulated after incubation with N23Ps. In conclusion, we bioinformatically selected IPF-relevant cytokines and identified them in cytokine arrays. Furthermore, we validated these cytokines using a transdifferentiation assay. Along with their corresponding downstream signalling molecules, these factors could potentially offer new therapeutic avenues for the treatment of lung fibrosis.

Contribution of a Disease-spanning Working Group: Single Cell Analysis

Abstract No. 404

HDAC9 deficiency drives senescence-associated impaired lipid metabolism during chronic inflammation in aged lung

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Introduction: Aging is characterized by progressive physiological changes that lead to senescence to adapt to metabolic stress in persistent low-grade pro-inflammatory state, influenced by epigenetic modification such as HDAC9. The aim of study is to unveil the role of HDAC9 deficiency leading to premature senescence phenotype.
Methods: In this study, we employed 2 different age groups. Stereleogical assessments, surfactant profiling and FACS were performed. Following, senescence associated phenotype was evaluated. RNA- and ATAC-Sequencing were employed with the following metabolome profiling. Finally, senolytic drug treatment in HDAC9 KO animals were performed.
Results: HDAC9 KO mice lungs displayed reduced alveolarization. β-gal stainings showed young HDAC9 deficient-AT2 cells developed senescent state along with increased p16 and p21 expressions and elevated pro-inflammatory cytokine releasing. iBALT formation was observed close to remodelled pulmonary vasculature. FACS analysis revealed that epithelial cells decreased in aged HDAC9 KO total lungs while $CD8^+/CD4^+$ T cell ratio significantly increased. Interestingly, HDAC9 deficiency led to reduced PCNA and SP-C positive cell numbers. Moreover, HDAC9 KO-AT2 cells showed reduced proliferation and increased apoptosis. In addition, ATAC and RNA seq-analysis unveiled that PLA2-related promoter regions were accesible, leading to upregulation of lipid related genes. Metabolomic profiling confirmed rewired choline metabolites into free fatty acid metabolites. Lastly, the number of β-gal positive cells decreased while pro-inflammatory genes were downregulated in HDAC9 KO lungs after senolytic drug treatment.
Conclusion: Targeting of HDAC9 is important for understanding the regulation of inflammaging-related lung remodeling.

Abstract No. 405

Bronchioalveolar lung organoids to model pulmonary fibrosis

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Pulmonary fibrosis is characterized by myofibroblast activation, tissue stiffening, and alveolar epithelial injury, with treatments to attenuate disease progression often failing. Given the need to develop novel therapeutic targets, organoid models have emerged as a powerful platform for disease modeling and drug screening. Here, we describe the use of bronchioalveolar lung organoids (BALO) to model distinct cellular and molecular characteristics of pulmonary fibrosis. BALO are generated from resident mesenchymal cells and bronchoalveolar stem cells and characterized by the formation of bronchiolar-like structures composed by club, ciliated, and basal cells and alveolar-like structures containing alveolar epithelial cells type I and type II (AECII), capable of surfactant secretion. The fibrotic BALO (fBALO) phenotype was TGF-β-induced over the course of 72 h, with increased numbers of α-SMA positive cells surrounding BALO being observed after 48 h of induction, using confocal microscopy. Fibrosis-related gene expression of *Col1a1*, *Acta2* and *Eln* was upregulated in a time-dependent manner. On the contrary, SPC^+ AECII were significantly reduced, with increased caspase 3 /7 indicating AECII apoptosis. To further study this model applicability, anti-fibrotics Nintedanib and Pirfenidone were used. Remarkably, only Nintedanib was able to revert the fibrotic phenotype, with a clear reduction of α-SMA myofibroblasts, observed and quantified by microscopy. Altogether, the TGF-ß-induced fBALO model can replicate features of the lung fibrosis phenotype, representing a promising tool to further analyze/develop novel anti-fibrotic drugs and to identify epithelial-mesenchymal interactions.

Abstract No. 406

Targeting IL6-trans and cis-signaling prevents vascular and bronchial remodeling in an experimental model of bronchopulmonary dysplasia

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Rationale Bronchopulmonary dysplasia (BPD) in the preterm infant is characterized by impaired alveolar and microvascular formation and a higher risk for pulmonary hypertension. We identified IL6 signaling being central in clinical and experimental BPD. Since inflammation is vital in the pathogenesis of pulmonary vascular and bronchial pathogenesis, we now investigated and targeted IL6-cis and -trans-signaling in experimental BPD.
Methods 1) Neonatal WT, IL6 knockout ($IL6^{-/-}$) mice and WT mice with pharmacological inhibition of IL6 cis (IL6 mAb) – and trans (sgp130Fc) signaling were exposed to hyperoxia (85% O_2, HYX) or normoxia (21% O_2, NOX). 2) Primary murine bronchial smooth muscle cells (pmBSMCs) were exposed to HYX and IL6/sIL-6 receptor (IL6+R), followed by assessment of viability and proliferation.
Results 1) WT^{HYX} mice showed vascular and bronchial wall thickening with reduced microvascular formation and a higher number of partly and completely muscularized microvessels (20-100 µm) when compared to WT^{NOX}. Moreover, the number of vascular $Ki67^+$ (marker of proliferation)/αSMA^+ cells and αSMA^+ cells per vessel were higher in WT^{HYX} than in WT^{NOX}. $IL6^{-/-}$ and inhibition of IL6 cis- and trans-signaling partly protected from hyperoxia-induced vascular and bronchial remodeling, but not from reduced microvascularization. 2) HYX decreased viability of pmBSMCs compared to NOX. While IL6+R reduced viability of pmBSMCs under NOX, it increased under HYX. Finally, IL6+R reduced the nuclear abundance of the anti-proliferative transcription factor FoxO1, resulting in proliferation of pmBSMCs exposed to NOX or HYX.
Conclusion Our data demonstrate IL6-cis and trans-signaling as a promising target to prevent vascular and bronchial remodeling through inhibition of SMCs proliferation.

Abstract No. 407

How informed are German patients with pulmonary sarcoidosis about their disease?

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Research question – Sarcoidosis is a multisystemic disease with a heterogenous course of disease. Comprehensive information about the complexity and treatment indications are essential for improving patient knowledge and adhering to therapy.
Objective – Aim of our study was to investigate the level and resources of information in patients with sarcoidosis, and to analyse differences in patient subgroups including age and gender.
Methods – We conducted a questionnaire-based online survey in Germany, and three semi-structured focus group interviews. The interviews were evaluated independently by two investigators using a structured qualitative content analysis.
Results – A total of 402 completed questionnaires were analysed, 65.8% of participants were women, and the mean age was 53 years. The majority of patients felt well informed about their disease in general (59.4%), but 40.6% were inadequately informed. The most relevant information gaps related to the future perspective (70.6%) as well as fatigue and diffuse pain (63.9%). Most patients received information from their treating pulmonologist (72.1%). 94% used the internet, especially homepages of patient support groups (75.2%). Male participants more often reported being well informed about their disease, and were more satisfied with the information (p= 0.001). During the interviews, patients expressed their wish for more comprehensive information, and highlighted the importance of psychological co-care as well as the future perspective.
Conclusions – A relevant proportion of patients with sarcoidosis are inadequately informed about their own disease, particularly with regard to factors impeding quality of life such as fatigue. Efforts are needed to improve the level and quality of information.

Abstract No. 408

Is the internet a sufficient source of information on Sarcoidosis?

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Research question – Many patients use the internet as source of health information. Sarcoidosis is a complex disease and internet resources have not yet been analysed for reliability and content.
Objective – The aim of our study was to investigate the content and the quality of information on sarcoidosis provided by internet resources.
Methods – Google, Yahoo and Bing were searched for the term “sarcoidosis” and the first 200 hits were saved in each case. Those websites that met the inclusion criteria (English language, no registration fees, relevant to sarcoidosis) were then analysed by two independent investigators for readability, quality, and content of the provided information.
Results – The websites were most commonly scientific or governmental (n= 57, 46%), and median time since last update was 24 months. Quality was rated with a median JAMA score of 2 (1; 4) and a median overall DISCERN score of 38 (SD 13), both scores represent partially sufficient information. 15% of websites had a HON certificate. Website content measured by median key fact score was 19 (range 2.5-25) with lowest scores for acute versus chronic course of disease, screening for extrapulmonary disease, and diffuse body pain. Worse results were achieved in industry websites and blogs (p= 0.047) with significant differences regarding definition (p= 0.004) and evaluation (p= 0.021).
Conclusions – Sarcoidosis related content of internet resources are partially sufficient; however, several important aspects are frequently not addressed and quality of information is moderate. Future directions should focus on providing reliable and comprehensive information on sarcoidosis; physicians from different disciplines and patients including self-support groups should collaborate on this.

Abstract No. 409

Deconvolution of bulk transcriptome profiles to discover cell type-specific IPF-associated genes and pathways

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Idiopathic pulmonary fibrosis (IPF) is a chronic lung disease in which **the scarring and stiffening of tissue leads to a decline in lung function. Transcriptome data contains cell mixtures and is confounded by differences in cell type proportions, and differentiating between regulated changes in gene expression and changes due to differing cell type proportions remains a challenge. To overcome this limitation, we apply a statistical deconvolution method to measure and correct for changes in cell type proportions.**

RNA-seq profiles of control (n=26) and IPF (n=46) lung samples from GSE134692 and control (n=103) and IPF (n=102) from GSE150910 and scRNA-seq data of control (n=28) and IPF (n=32) samples from GSE136831 were considered. MuSiC was used for deconvolution. The first 4 principal components of cell type proportions were used for correction, followed by pathway analysis.

Inferred cell type proportions showed a significant increase in ciliated cells, fibroblasts, and a decrease in ATI cells. Cell type proportion correction resulted in a reduction of genes associated with IPF. Pathway analysis after this correction retained the expected findings of TGF-β signaling and extracellular matrix pathways but also indicated the activation of NF-kappaB, which was otherwise missed. We also develop a method for assessing IPF pathways in a cell type-specific manner in bulk data. This novel technique implicates the role of interferon signaling in ATII cells.

Deconvolution is an efficient way of increasing the utility of bulk transcriptome data. This method shows promise performing cell type-specific pathway analysis on bulk data in the context of IPF as well as other disease areas in DZL.

Abstract No. 410

The impact of neonatal hyperoxia on iNOS signaling and consequences in adulthood

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Background: Bronchopulmonary dysplasia (BPD) is a chronic lung disease of premature infants caused mainly by oxygen supplementation and/or mechanical ventilation. BPD is characterized by arrested alveolarization. Nowadays BPD is not fully understood and the long-term consequences in adulthood are not well known. Evidence suggests that inducible nitric oxide synthase (iNOS) may play a role in BPD and in the susceptibility to develop chronic lung diseases (CLDs) in adulthood. Therefore, we hypothesized that iNOS might be involved in the development of BPD and CLDs in adulthood.
Methods: C57BL/6J newborn mice have been exposed to room air (21% O_2) and to hyperoxia (85% O_2) from postnatal day (P)1 to P14. Gene expression analysis were assessed in P14 lung homogenates by real-time RT-PCR. Single-cell RNA sequencing (scRNA-seq) data of P14 mouse lung homogenates exposed to 21% O_2 and 85% O_2 were analyzed to establish the expression of *Nos2* in specific cell clusters of the lung (1).
Results: Gene expression analysis showed an increase of *Nos2* transcripts in the lung homogenate from P14 mice exposed to 85% O_2 compared to mice exposed to 21% O_2. scRNA-seq analysis revealed that *Nos2* is expressed in Foxj1$^+$ ciliated cells and in Car4$^+$ aerocytes (aCap). Interesting, hyperoxia reduced the expression of *Nos2* in Foxj1$^+$ ciliated cells and increased *Nos2* expression in aCap.[1]
Conclusion: These data reveal a hitherto unknown role of *Nos2* in experimental BPD. Moreover, the impact of hyperoxia on *Nos2* transcript levels in Foxj1$^+$ ciliated cells and aCap might play a crucial role in arrested alveolarization with consequences in adulthood. Further studies are necessary to clarify the role of iNOS in Foxj1$^+$ ciliated cells and aCap *in vivo*.

Contribution of a Disease-spanning Working Group: Lung Environment Interaction

References:
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Abstract No. 411

Role of miR-29c in extracellular matrix deposition during lung alveolarization

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Preterm babies often require supraphysiological oxygen conditions in order to ensure survival. This can lead to the development of bronchopulmonary dysplasia (BPD), a disease where alveoli are fewer and larger, the gas-exchange surface area is reduced and the pulmonary circulation is dysmorphic. One of the pathogenic mechanisms involved in this disease is the disbalance in deposition of extracellular matrix (ECM) components such as collagen and elastin. In this study, a small non-coding RNA, miR-29c, is hypothesized to play a causal role in the generation of disturbed ECM in the affected lungs.
Bronchopulmonary dysplasia can be modelled experimentally by exposing newborn mice to high oxygen concentrations. A microarray analysis of the gene expression in lungs of mice exposed to 85% O_2 for 14 days (P14) revealed an increase in miR-29c abundance, and RT-PCR analysis confirmed this abundance at P3, P5 and P14. Genetic ablation of mir-29c-3p with an antimir partially preserved alveolar development under hyperoxia, validating a pathogenic role for miR-29c in aberrant lung development. Fluorescent *in situ* hybridization of mice lungs revealed that expression of miR-29c takes place mainly in the mesenchyme, suggesting a regulatory role in fibrogenesis. An *in silico* analysis of miR-29c targets suggests that both ECM components and ECM remodelling enzymes are potentially regulated by miR-29c. Some of those targets were validated by RT-PCR and western blot using primary fibroblasts transfected with a miR-29c-3p mimic.
These data highlight miR-29c as a pathogenic mediator of aberrant lung development due to oxygen toxicity. Studies currently underway include the transgenic ablation of miR-29c *in vivo* and the proteomic analysis of antimir treated lungs.

Abstract No. 412

A spatially resolved proteome atlas of the distal human lung

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The pulmonary extracellular matrix (ECM) provides structural integrity and essential mechanical properties such as elastic recoil, but also serves as an information-rich signaling template that instructs cell identity and activity in health and disease. The spatial distribution and specificity of ECM proteins to the lungs distinct tissue niches is mostly unknown.
In this study, we use spatially resolved mass spectrometry-based proteomics to define global differences in the proteomic composition of anatomically and histologically defined regions of the distal human lung. We characterized the distal airway tree and associated arteries and veins from 12 human donors*, using both 3D surgical microdissection (n=5) and 2D laser-capture microdissection techniques (n=7). Our analysis identified 7594 proteins (including 446 matrisome proteins) from 3D dissections and 4898 proteins (including 341 matrisome proteins) from 2D dissections.
We identified gradients of ECM composition along the proximal-distal axis of the airways and vessels and discovered a specific ECM and immune niche in the respiratory bronchioles (RB). Integrative analysis of the spatial proteomes with donor-matched snRNAseq data and the Human Lung Cell Atlas (HLCA) further suggests the presence of a novel RB-associated fibroblast state that can be found across donors also in public datasets. As proof of concept, we have identified and validated a subset of fibroblasts characterized by an ECM expression program enriched in RB. Given the potential origin of chronic lung diseases in distal airways, further investigation of this novel RB specific ECM niche would provide more insights into lung homeostasis and pathophysiology.
*: Received after informed consent.

Contribution of a Disease-spanning Working Group: Single Cell Analysis

Abstract No. 413

LC3B interactome in alveolar epithelial cells: novel insights of autophagy regulation in lung fibrosis

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Autophagy is a dynamic intracellular catabolic process and a quality control mechanism that maintains cellular homeostasis by degrading long-lived proteins or damaged organelles. It is facilitated by a concerted action of several autophagy-related proteins that convert the cytosolic form of Microtubule-associated protein 1 light chain, subunit 3 (LC3BI) to the membrane-bound LC3BII. Altered autophagy is implicated in several diseases including idiopathic pulmonary fibrosis (IPF) and animal models of lung fibrosis. We previously demonstrated the localization of LC3B to lamellar bodies of the alveolar epithelial type II cells (AECII) and its interaction with Cathepsin A (Cat-A), a lysosomal protective protein. We aimed to identify (1) the LC3B-Cat-A interaction via its LIR and (2) novel interactors for LC3B. Five potential LIRs in Cat-A were found via in silico analysis and we performed site-directed mutagenesis to incorporate point mutations. We generated Myc-tagged constructs alongside full-length Cathepsin A and we observed a decreased Cat-A - LC3B interaction only in cells overexpressing LIR1 (W149D/L152H) as compared with wt Cat-A. In addition, we performed shot-gun proteomics for endogenous LC3B in AECII and identified Syndecan-4 (Sdc4), a transmembrane heparin sulphate proteoglycan as a novel LC3B interactor. The LC3B-Sdc4 interaction was established via immunoprecipitation. Immunofluorescence images indicate a cytosolic and membrane-bound localization of Sdc4 in alveolar epithelial cells. The expression of Sdc4 was also found elevated in IPF & DO lung homogenates. Overall our data open up new avenues for LC3B regulation in eliciting cellular stress pathways and subsequently in the development of lung disorders.

Abstract No. 414

The role of niche-specific epithelial-mesenchymal crosstalk in human fibrogenesis

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Epithelial-mesenchymal crosstalk (EMC) has proven to be essential in lung development and repair upon injury. Such crosstalk occurs in specific subtissular niches, in which niche-specific epithelial cell states interact with their mesenchymal counterpart driving alveologenesis or re-epithelialization, respectively. IPF is characterized by a fulminant profibrotic niche, the fibroblast focus, in which CTHRC1+ myofibroblasts (CMFs) reside in close contact with aberrant basaloid cells (abBC). However, it is unknown how these fibrosis-specific cell states evolve from their healthy relatives, probably through various preceding injury associated profibrotic cell states (IAPCS). In this study, we aim to identify putative preceding IAPCS, locate the distinct niches in which they reside and study how the niche-specific EMC drives the epithelial and mesenchymal transdifferentiation (EMTD) into CMFs and abBCs, respectively.

Ninety IPF explant lung samples were microCT-staged in mild, moderate and severe and additionally compared with 45 control samples, enabling to study progression of human fibrogenesis. Using snRNAseq, laser-capture microdissection-based spatial proteomics (LCMD-SP) and iterative indirect immunofluorescent imaging (4i), we identified a spectrum of IAPCS residing in distinct niches with unique proteome profiles. Cell-cell communication analysis using Nichenet showed niche-specific ligand-receptor interactions driving each EMTD step with several ligands prioritized by Nichenet enriched in the respective niche.

In conclusion, using our multimodal approach, we identified multiple subtissular niches within the fibrotic lung in which EMTD was clearly recapitulated. Cell-cell communication analysis showed the importance of the niche as a driver of this process.

Contribution of a Disease-spanning Working Group: Single Cell Analysis

Abstract No. 415

An In Vitro Mini-Lung IPF Model with Real-Time, Non-Invasive Monitoring of Compliance as a Functional Fibrosis Biomarker

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The shortage of predictive preclinical fibrosis models is one of the main challenges in idiopathic pulmonary fibrosis (IPF) drug development. We developed an advanced *in vitro* "breathing" mini-lung fibrosis model consisting of i) a triple primary-based coculture model of the alveolar tissue comprised of endothelial, epithelial cell lines combined with primary fibroblasts derived from IPF patients, ii) a novel millifluidic cell-stretch CIVIC bioreactor system, and iii) a bioinspired BETA basement membrane. The millifluidic system also allows medium (blood) perfusion, metered aerosolized drug delivery, and real-time, non-invasive lung function (compliance) monitoring as a functional diagnostic marker of fibrosis. The BETA basement membrane has unique alveolar-like properties, including low thickness (<1 µm) and high biocompatibility, permeability, and elasticity (<10 kPa) for culturing of multiple cell types under air-liquid interface (ALI) and cyclic mechanical stretch conditions. Leveraging this patented BETA/CIVIC mini-lung technology, we investigated the drug efficacy of inhaled and systemically (oral) delivered Nintedanib under cell stretch conditions. Nintedanib shows more potent anti-inflammatory (IL1β, IL-6, and IL8) and anti-fibrotic (αSMA, soluble and deposited (type I) collagen, and compliance) effects under cell stretch conditions for both routes of delivery. We demonstrate that cyclic mechanical stretch enhanced the Nintedanib-induced restoration of alveolar phenotypes, as indicated by surfactant protein C and Yes-associated protein levels. Our bioinspired *in vitro* mini-lung model improves the functional (clinical) relevance of *in vitro* fibrosis models and provides a platform for combined longitudinal mechanistic, pharmacodynamics, and -kinetics studies.

Abstract No. 416

Pericyte-derived myofibroblasts in murine lung fibrosis

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Research question: Pulmonary fibrosis is a chronic and fatal disease accompanied by the formation of α smooth muscle actin (αSMA)-positive myofibroblasts. These myofibroblasts are decisively involved in the increased production of extracellular matrix leading to scarring and reduced lung function. Among other cell types, pericytes are discussed as myofibroblast progenitors. Due to the lack of unique markers, unambiguous identification of pericytes is challenging. In addition, the unequivocal proof that myofibroblasts originate from pericytes is still missing in murine lung fibrosis.

Objective: Aim of the current study was to clarify whether pericytes act as myofibroblast precursors.

Methods: Immunofluorescence analysis was used to identify pericytes and myofibroblasts. Tamoxifen-inducible lineage tracing of pericytes was performed using mice expressing the fluorescent dye tdTomato under the platelet-derived growth factor receptor β (PDGFRβ) promotor. Lung fibrosis was induced by a single orotracheal dose of bleomycin (2 U/kg) and fibrotic changes were assessed after 21 days. Lung tissue from untreated animals served as control.

Conclusion: We demonstrate that pulmonary pericytes can be labeled by the combination of NO-sensitive guanylyl cyclase (NO-GC) and PDGFRβ. In addition, pericytes can be effectively traced by PDGFRβ-tdTomato in murine lung. In fibrotic lung, lineage tracing experiments show that a small subgroup of myofibroblasts derive from pericytes. Open-source single-cell sequencing data of human lung tissue from patients with pulmonary fibrosis reveal a αSMA/NO-GC/PDGFRβ-positive myofibroblast subtype suggesting the transferability of our data to humans. Consequently, pericytes might play an essential role in the pathogenesis of interstitial lung disease.

Abstract No. 417

Functional profiling of primary human fibroblasts derived from healthy and diseased lung tissue

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Chronic lung diseases contribute significantly to the health burden world-wide. Primary human lung fibroblasts (phLFs) isolated from healthy and diseased tissue are frequently used to investigate fibroblast behavior in chronic obstructive pulmonary disease (COPD) and interstitial lung disease (ILD).

Here, we investigate the disease-relevant functional differences in age- and sex-matched phLFs derived from healthy, COPD, idiopathic pulmonary fibrosis (IPF) and non-IPF-ILD tissue. Using an established high-content ECM deposition assays and additional α smooth muscle actin stainings with those phLFs, we identified functional differences in proliferation, fibroblast to myofibroblast transdifferentiation and ECM deposition, and correlated these with clinical patient characteristics. While both COPD and IPF phLFs showed a increased deposition of disease-relevant Collagen I and V compared to healthy controls, non-IPF phLFs exhibited only an increase in Collagen V deposition. αSMA expression was increased in COPD- and non-IPF-ILD-derived phLFs in response to TGFβ1, but not in non-IPF-ILD-derived phLFs. Next, comparing the observed functional differences with clinical parameters demonstrated that Collagen I and V deposition were firmly correlated with patients' age (R^2=0.62 and R^2=0.44 respectively) across disease origin, whereas Collagen V deposition was correlated only with donor smoking history.

In conclusion, our study identified pivotal diversities in disease-relevant fibroblast functions that should be considered for the design of future pathophysiological in vitro studies as well as drug discovery assays.

Abstract No. 418

Ex vivo tissue perturbations coupled to single cell RNA-seq to study multi-lineage cell circuit dynamics in human lung fibrogenesis

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Pulmonary fibrosis develops as a consequence of failed regeneration after injury. Analyzing mechanisms of regeneration and fibrogenesis directly in human tissue has been hampered by the lack of organotypic models and analytical techniques. In this work, we coupled *ex vivo* cytokine and drug perturbations of human precision-cut lung slices (hPCLS) with scRNAseq and induced a multi-lineage circuit of fibrogenic cell states in hPCLS, which we show to be highly similar to the *in vivo* cell circuit in a multi-cohort lung cell atlas from pulmonary fibrosis patients. Using micro-CT staged patient tissues, we characterized the appearance and interaction of myofibroblasts, an ectopic endothelial cell state and basaloid epithelial cells in the thickened alveolar septum of early-stage lung fibrosis. Induction of these states in the *ex vivo* hPCLS model provides evidence that the basaloid cell state was derived from alveolar type-2 cells, whereas the ectopic endothelial cell state emerged from capillary cell plasticity. Cell-cell communication routes in patients were largely conserved in the hPCLS model and anti-fibrotic drug treatments showed highly cell type specific effects. We have established siRNA mediated genetic perturbations on this platform and are currently dissecting cell differentiation trajectories using multimodal and multiplexed time resolved single cell genomic profiling. Our work provides an experimental framework for perturbational single cell genomics directly in human lung tissue that enables analysis of tissue homeostasis, regeneration and pathology. We further demonstrate that hPCLS offers novel avenues for scalable, high-resolution drug testing to accelerate anti-fibrotic drug development and translation.

Abstract No. 419

Spatiotemporal analysis of inflammaging and senescence programs in lung fibrosis using single-cell transcriptomics and multiplexed immunofluorescence

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Aging is a major risk factor for chronic lung disease such as pulmonary fibrosis. While bleomycin injury in young mice leads to resolving fibrosis, aged mice develop non-resolving fibrosis, resembling human disease.

Here, we conducted a longitudinal single-cell RNA profiling of young (3 mo) and aged (18 mo) mice after bleomycin-induced lung injury (days 0/3/10/20/30/37 p.i., n=55 mice), coupled to multiplexed immunofluorescence to resolve cellular programs responsible for this impaired regeneration.

Single-cell analyses of over 160,000 cells across 69 cell states combined with imaging revealed key differences in cell type abundances, gene expression and cell-cell communication in aged vs young mice at baseline and during injury and repair. Aged mice showed a significant change in the lymphocyte repertoire, including the emergence of pro-inflammatory B and T-cell populations. We observed a persistent alveolar-mesenchymal crosstalk and sustained fibroblast activation driven mainly by epithelial Krt8+ alveolar differentiation intermediates. This was accompanied by increased and prolonged cellular senescence and DNA damage. Digital sorting of senescent alveolar cells based on the canonical senescence genes showed an age-specific gene expression and SASP profile, including Macrophage migration inhibitory factor (MIF). MIF was predicted to cause fibroblast activation in aged mice, which was confirmed by *in vitro* MIF stimulation of mouse fibroblasts.

Our study provides a comprehensive single-cell map of the molecular changes of age-related lung fibrosis and identifies inflammaging and persistent senescence as crucial pathogenic mechanisms.

Contribution of a Disease-spanning Working Group: Single Cell Analysis

Abstract No. 420

Susceptibility to viral infection driving lung injury and disruption of lung development in neonates with bronchopulmonary dysplasia

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Bronchopulmonary dysplasia (BPD) is a common complication of preterm birth with significant morbidity and mortality, and is experimentally modelled by exposure of newborn mice to hyperoxia to mimic oxygen supplementation of preterm infants. Oxygen toxicity creates a permissive environment for respiratory virus infection that exacerbates stunted post-natal lung maturation, by as-yet unclarified mechanisms. Those mechanisms will be explored using a hyperoxia-based experimental mouse model of BPD ("experimental BPD"), together with infection with pneumonia virus of mice (PVM).
Changes in lung inflammatory cell dynamics in juvenile C57BL/6J mice exposed to normoxia (21% O_2) and hyperoxia (85% O_2), with or without PVM, for the first 10 days of post-natal life were assessed by single cell RNA sequencing (scRNA-Seq) of a $CD45^+$ $Ly6G^-$ lung cell population. Chromatin accessibility was investigated by batch assay for transposase-accessible chromatin (ATAC)-Seq analysis, and validated by batch RNA-Seq. Resident alveolar macrophages and lymphocytes were measured by FACS at postnatal day 14.
Hyperoxia exposure in combination with PVM yielded poorer survival, a lung environment permissive for PVM replication and very distorted lung architecture. There was also an exacerbated loss of resident alveolar macrophages, and differences in lymphocyte populations. Single cell RNA-Seq analyses revealed changes in inflammatory cell clusters.
Oxygen support increased the permissiveness of the lung for PVM infection. Oxygen toxicity combined with PVM infection worsened of lung development. In oxygen-injured lungs, resident alveolar macrophages were identified to be pathogenic mediators of arrested lung development and combined with PVM infection were decreased in abundance.

Abstract No. 421

Carbon nanoparticle-triggered gammaherpesvirus reactivation in the lung

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Two ubiquitous factors challenge our lung continually, lifelong latent herpesvirus infection and inhalation of environmental particles. Our group has shown that pulmonary exposure to carbon nanoparticles (CNP) can trigger reactivation of latent murine gammaherpesvirus 68 (MHV-68) infection in mouse lungs. Reactivation was mainly localized to $CD11b^+$ infiltrating macrophages and dependent on p38 MAPK signaling. Further, we observed that a two-time repeated CNP administration to latently infected mice resulted in alveolar damage leading to progressive emphysema.
The underlying adverse mechanisms causing alveolar damage and the corresponding cell communication upon CNP exposure in latently infected lungs are still unclear and subject of my current project.
To have an *in vitro* model at our disposal, I developed a 3D lung organoid culture from primary murine epithelial cells in combination with persistently MHV-68 infected macrophages. I could already show that virus reactivation and transcription of cytokines, e.g. Cxcl1 and Csf2, are inducible in this setting. With this, I hope to gain insights into the cellular communication between epithelial cells and macrophages and to understand the mechanisms underlying reactivation-triggered damage.
In addition, I will investigate adverse effects on epithelial cells and disease development *in vivo*. To this end, latently infected mice will be exposed to CNP up to 5 times, and virus reactivation and further epithelial damage will be analyzed.
By uncovering molecular mechanisms underlying the adverse effects and elucidating communication occurring *in vitro* and *in vivo*, I want to identify potential targets to prevent exacerbation of lung diseases caused by these two omnipresent factors, herpesvirus infection and ambient particle exposure.

Abstract No. 422

Lung single cell transcriptomics to guide the development of AOP anchored cell-based assays in response to nanomaterial exposure

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Inhaled nanomaterials (NM) can cause acute and chronic inflammation. Here we seek to identify NP-specific cellular perturbation pathways and the underlying key cell types to inform adverse outcome pathway (AOP) anchored *in vitro* studies, by single cell transcriptomics. We exposed mice intratracheally to carbon black (CNP), tangled double-walled (DWCNT) and rigid, multi-walled carbon nanotubes (MWCNT). All lungs underwent single-cell RNA sequencing (scRNA-seq), histology and BAL analysis.
At the chosen doses all NMs caused comparable airspace neutrophilia at 12h, which increased until d6 for the CNTs but remained elevated until d28 only for MWCNT, indicating the key event (KE) acute and chronic inflammation. Comparing scRNA-seq and BAL cytokine levels at 12h, the fate of inflammation was characterized in a NM-specific manner. In agreement with airspace neutrophilia, CNP uniquely triggered GM-CSF and CXCL1 release, with Csf2 and Cxcl1 mRNAs being expressed mainly in alveolar epithelial cells. DWCNT caused CCL2, -3 and -4 release involving interstitial macrophages and monocytes, associated with acute high lung monocyte and at d6 high BAL macrophage numbers. MWCNT in contrast caused ample BAL cytokine increases involving different cell types and specifically caused a Th2 cytokine response (CCL11, IL10), a crucial KE of fibrosis AOPs. MWCNT triggered cell communication analysis uncovered NM-specific networks including epithelial cells, fibroblasts, macrophages, dendritic cells and endothelial cells.
Our study uncovers early NM-specific cell perturbations and identified first specific cellular response pattern, more complex cell-cell communications might have to be considered to recapitulate KE response pattern to guide AOP predictive cell-based testing strategies.

Contribution of a Disease-spanning Working Group: Lung Environment Interaction

Abstract No. 423

OMIC-Informed Bronchopulmonary Dysplasia Endotypes Reveal Signatures of Adult Chronic Lung Disease

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Bronchopulmonary Dysplasia (BPD) is a common long-term complication in **premature infants resulting from impaired development of the gas-exchange area. Molecular mechanisms underlying BPD development and its potential link to adult chronic lung diseases (CLD) are incompletely understood. We compiled a BPD multi-omics study cohort (n= 55 preterm infants < 32 weeks gestational age) and analyzed 1120 proteins, 213 metabolites, and 62 critical clinical variables to identify major disease endotypes through applying multi-factor analysis. We successfully identified 5 major BPD endotypes characterized by OMIC expression indicating 1) lung surfactant deficiency; 2) coagulation and vascular remodeling; 3) metabolic dysregulation; 4) developmental growth factor signaling; and 5) signal transmission including stress response and synaptic signaling. We performed t-test and random forest to establish specific OMIC 'susceptibility' signatures of the BPD endotypes that can be traced into OMIC pattern of adult CLD (Chronic Obstructive Pulmonary Disease (COPD), n=21; Interstitial Lung Disease (ILD), n=20). BPD endotypes 4 and 5 showed a close OMIC pattern correlation with COPD, including consistent up-regulation of proteins such as FYN and IF4G2. In addition, we revealed the correlated regulation of distinct proteins with ILD, including SELE and CAPG.**
In summary, we successfully identified biologically defined BPD categories and their effective connection with OMIC patterns of adult CLD. We expect our results to inform the search for clinically relevant markers that can improve the diagnostic and therapeutic process in BPD patients.

Abstract No. 424

Collagen prolyl-3-hydroxylase 1 controls collagen modification and extracellular matrix deposition

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Rationale: Collagen prolyl-3-hydroxylase 1 (P3H1) catalyses 3-hydroxylation of specific collagen sites and is one of the most consistently upregulated proteins in idiopathic pulmonary fibrosis (IPF). We hypothesized that inhibiting *P3H1* expression changes the collagen post-translational modification (PTM) profile and alters extracellular matrix (ECM) deposition.
Objective: To identify and quantify changes in collagen PTMs and ECM profiles under conditions of P3H1 deficiency.
Methods: We assessed *P3H1* expression by immunofluorescent stainings and immunoblot analysis of IPF samples. We knocked down P3H1 using siRNA in primary human bronchial epithelial cells (phBEC) and lung fibroblasts (phLF), and analysed gene expression by qPCR and Western Blot analysis, and the matrisome by MS/MS-based proteomics. Using amino acid analysis (AAA) and a bioinformatic MS/MS analysis pipeline established by us [1], we quantified type I collagen PTMs in fibroblast-derived ECM and in purified type I collagen extracted from P3H1 knockout mice.
Results: P3H1 was expressed by epithelial cells and fibroblasts in IPF lung sections. P3H1 knockdown in phBEC resulted in a loss of basement membrane ECM (laminins, type IV and XVII collagen) and an increase of type I collagen expression. In phLF, P3H1 knockdown lead to an overall increase of deposited ECM including fibrillar collagens, tenascin, and fibronectin. Finally, P3H1 deficiency resulted in prolyl and lysyl overhydroxylation and lysyl overglycosylation at many sites.
Conclusions: P3H1 regulates collagen modification and extracellular matrix deposition far beyond changes in prolyl-3-hydroxylation and collagen composition and may counteract excessive ECM deposition in lung fibrosis.
References:
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Abstract No. 425

The Netrin1-Klf4 axis as an integrator of lung fibroblast homeostasis and a target for bronchopulmonary dysplasia and idiopathic pulmonary fibrosis

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Rationale: Lungs with bronchopulmonary dysplasia (BPD) or with idiopathic pulmonary fibrosis (IPF) are characterized by activated fibroblasts and matrix remodeling. We recently identified Krüppel-like factor 4 (Klf4) as a key transcription factor in fibroblast biology and to be an effector gene of Netrin1 in alveolar epithelial type 2 cells. We now studied the Netrin1 (Ntn1)-Klf4 axis in fibroblasts in BPD and IPF to derive new anti-fibrotic therapeutic strategies.
Methods: (i) Assessment of Ntn1, its receptor Unc5b and Klf4 in hyperoxia-exposed neonatal mouse lungs and lungs of individuals with IPF. (ii) Genetic and pharmacological modulation of Ntn1 and Klf4 in primary neonatal murine lung fibroblasts (pnF) and fibroblasts from human IPF lungs. (iii) Ntn1 null mice and wildtype mice treated with Netrin1. (iv) Engineering a delivery system for Klf4 using nanoparticle technology.
Results: (i) Ntn1, Unc5b, and Klf4 were markedly downregulated in neonatal mouse lungs after hyperoxia and in primary IPF fibroblast. (ii) Chip-Seq and biotin proximity identification assay revealed a profibrotic profile of Klf4 and its role in acetylation processes. In pnF and IPF fibroblasts, Klf4 had an antiproliferative, pro-apoptotic, and migratory effect. Treatment with Ntn1 increased Klf4 in fibroblasts from lungs with IPF by 200%. (iii) *In vivo* studies using Ntn1 null mice and wildtype mice treated with Ntn1 confirmed Netrin1 as a regulator of collagen deposition and αSMA^+ lung cell population. (iv) Thus, we now developed a liposomes-based Klf4 delivery nanoparticle as an antifibrotic strategy.
Conclusion: Our study demonstrate a Ntn1-Klf4 axis in lung fibroblasts as a possible mechanism of fibrotic processes and lung growth arrest in BPD and IPF, thus offering new therapeutic avenues.

Abstract No. 426

Inflammaging modulates ATII cell senescence in pulmonary fibrosis

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Idiopathic Pulmonary Fibrosis is a chronic lung disease predominantly affecting the elderly, suggesting that cellular aging including cellular senescence predisposes to disease. “Inflammaging” is a chronic inflammatory state commonly observed in aging, due in part to deficient senescent cell clearance. Pharmacological targeting of senescent cells by senolytics ameliorates lung fibrosis in young mice, however, its effects on aged fibrotic lungs are unknown.

Here, we performed bulk RNA sequencing on young and old senolytic-treated precision cut lung slices (PCLS) obtained from mice after bleomycin-mediated fibrosis. We evaluated how senolytics modulate gene expression and key biological processes involved in fibrosis. Significant changes in Granzyme K (GZMK) and IL6 indicate a shift in the immune response post-treatment. Gene set enrichment analysis showed a decrease in overall activity of multiple immune pathways especially in aged PCLS upon senolysis.

Co-culture of age-associated T-Cells producing Granzyme K with primary mouse alveolar epithelial type (AT)II cells reduced ATII progenitor cell potential. Mechanistically, GZMK treatment induced cellular senescence in ATII cells as assessed by RT-qPCR and senescence-associated ß-galactosidase staining. It thereby limits regenerative capacity of ATll cells in an organoid assay. GZMK induced a senescence-associated secretory phenotype (SASP) characterized by macrophage inhibitory factor (MIF) in vitro, which leads to fibroblast activation, a fibrosis hallmark.

We present a putative mechanism linking inflammaging to cellular senescence of ATII cells and increased fibroblast activation. We will further expand our studies into the cross-talk between ATII cells and age-associated adaptive immune cells in the context of fibrosis.

Contribution of a Disease-spanning Working Group: Lung Environment Interaction

Abstract No. 427

RNA – Autophagy interplay in diffuse parenchymal lung diseases

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RN autophagy is a cellular quality control mechanism in which RNA is taken up by lysosomes for degradation in an ATP-dependent manner. RNA-binding proteins (RBPs) associate with target ribonucleic acids (RNAs) through conventional or unconventional RNA-binding domains to regulate all aspects of messenger RNA (mRNA) biogenesis and metabolism. In organs with complex RNA metabolism such as the brain or lung, the role of RBPs becomes even more important. The aim of this study is to determine the interaction between autophagy and RNA quality control and to understand how this knowledge contributes to the development of diffuse parenchymal lung diseases (DPLDs). In general, differential regulation of several RBPs including TDP43, FUS, SRF, MBNL1, and PABPC1 was observed in the lungs of patients with idiopathic pulmonary fibrosis (IPF). Since our previous studies showed that the autophagy-associated protein, LC3B and LC3 B-dependent autophagy plays a protective role in the context of lung fibrosis, we performed RNA immunoprecipitation assays to detect novel RNAs that are bound to LC3B in both alveolar epithelial cells as well as in interstitial fibroblasts. Of note, we observed that the mRNAs namely *Mars1, Prmt1,* and *fibronectin* bind to LC3B in alveolar epithelial cells. Our readouts so far implicate a critical role for LC3B-dependent RNA quality control in normal lung health and under conditions of lung fibrosis.

Abstract No. 428

Dextromethorphan inhibits collagen transport in the endoplasmic reticulum eliciting an anti-fibrotic response in *ex-vivo* and *in vitro* models of pulmonary fibrosis

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Despite our understanding of the molecular mechanisms underlying pulmonary fibrosis, a cure for pulmonary fibrosis has not yet been found. To this end, in this study, we screened an FDA-approved drug library containing 712 drugs and found that Dextromethorphan (DXM), a cough expectorant, significantly reduces the amount of excess fibrillar collagen deposited in the ECM in *in-vitro* cultured primary human lung fibroblasts (NHLF) and *ex-vivo* cultured human precision-cut lung slice (hPCLS) models of lung fibrosis. Reduced extracellular fibrillar collagen levels in the ECM upon DXM treatment are due to a reversible trafficking inhibition of collagen type I (COL1) in the endoplasmic reticulum (ER) in TANGO1 and HSP47 positive structures. Mass spectrometric analysis shows that DXM causes hyper-hydroxylation of proline and lysine residues on Collagen (COL1, COL3, COL4, COL5, COL7, COL12) and Latent-transforming growth factor beta-binding protein (LTBP1 and LTBP2) peptides coinciding with their secretion block. In addition, target engagement analysis using thermal proteome profiling of cells treated with DXM, shows increased thermal stability of prolyl- hydroxylases such as P3H2, P3H3, P3H4, P4HA1 and P4HA2. Transcriptome analysis of pro-fibrotic stimulated NHLFs and hPCLS upon DXM treatment showed activation of an anti-fibrotic program via regulation of pathways such as those involved in the MMP-ADAMTS axis, WNT, and fibroblast-to-myofibroblast differentiation. Taken together, the data obtained from both in-vitro and ex-vivo models of fibrogenesis show that Dextromethorphan has potent anti-fibrotic activity by efficient inhibition of COL1 membrane trafficking in the ER.

Abstract No. 429

Conditional overexpression of Chop in alveolar epithelium protects transgenic mice from lung fibrosis induced by bleomycin

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Rationale: Idiopathic pulmonary fibrosis (IPF) is an uncurable interstitial lung disease. The irreversible scarring of the lung leads to a progressive loss of respiratory function. Although the origin is still unknown, a maladaptive endoplasmic reticulum (ER) stress-response in type-II alveolar epithelial cells (AECII) has been identified as its main characteristic.
Objectives: Since the upregulation of the proapoptotic ER stress-factor C/EBP homologous protein (CHOP) in AECII is a hallmark of IPF, we decided to investigate its role in the development of age-related as well as bleomycin-induced lung fibrosis, by using a transgenic mouse line with conditional AECII-specific overexpression of Chop based on the Tetracycline-On/Off-system [SP-C rtTA/(o-tet7)-Chop].
Methods: Chop-transgene expression was induced in young (3 mo) and aged mice (12-24 mo) through doxycycline (Dox+) feeding for 14 days, followed by sacrifice and phenotyping of mice. We also treated young "Chop-mice" intratracheally with bleomycin after 14 days (Dox+) feeding, and compared them to two control groups at different post-application time-points.
Results: Despite successful nuclear Chop overexpression in very aged transgenic mice, there was no pronounced development of lung fibrosis in these mice. Further, transgenic mice overexpressing Chop in the AECII did not indicate greater AECII apoptosis and aggravated lung fibrosis than transgenic mice without Chop induction following bleomycin-treatment. Instead, markers of apoptosis and fibrosis were significantly reduced in the Chop expressing mice at days 14 and 28 post bleomycin, in comparison to bleomycin-mice without Chop induction.
Conclusions: Mice with conditional AECII-specific overexpression of Chop are protected from bleomycin-induced lung fibrosis.

Abstract No. 430

Increased susceptibility to lung fibrosis by gammaherpesvirus infection in transgenic mice with conditional overexpression of the ER stress-factor Chop in alveolar epithelium

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Rationale: IPF is characterized by ER stress and apoptosis in type-II alveolar epithelial cells (AECII). We developed transgenic mice expressing the proapoptotic ER stress-factor Chop exclusively in AECII by using the Tetracycline-On/Off-system [SP-C rtTA/$(tetO)_7$-Chop]. In response to doxycycline-feeding (Dox+) for 28 days, nuclear Chop-overexpression in AECII resulted in AECII apoptosis, but not in development of lung fibrosis, despite upregulation of profibrotic markers.
Objective: We suggested that, similar to human IPF, the '*Chop* mice' reflect individuals with vulnerable AECIIs prone to develop lung fibrosis in response to second hits. Among these, herpesvirus infection has received great attention as an etiologic factor of IPF, as a higher prevalence of herpesviruses was identified in lungs of IPF-patients.
Methods: We infected transgenic mice 28 days after transgene-induction with murine gammaherpesvirus-68 (MHV68), and harvested the lungs 15 days post infection.
Results: MHV68 infected Dox+ *Chop* mice, but not infected Dox- transgenic mice fed with standard chow or uninfected Dox+ and Dox- controls, demonstrated significant development of lung fibrosis. Viral genomic load was higher in lungs of infected Dox+ than Dox- mice. In addition, apoptosis was observed in AECII and alveolar macrophages (AM) of both MHV68 infected Dox+ and Dox- transgenic mice versus uninfected controls, but was more increased in the infected Dox+ *Chop* mice. Further, Dox+ *Chop* mice infected with MHV68 revealed increased M2 macrophage polarization versus infected Dox- mice.
Conclusion: Targeted AECII injury by ectopic Chop expression promoted chronic MHV68 infection in transgenic mice and also rendered the lung to increased susceptibility to M2 macrophage activation, ending up in lung fibrosis.

Abstract No. 431

Using *in vivo* microcomputed tomography for monitoring IPF-like disease in conditional *Nedd4-2* deficient mice

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Objectives: Idiopathic pulmonary fibrosis (IPF) is a fatal lung disease which is usually diagnosed in advanced stages. Little is known about the subclinical development of IPF. A novel mouse model with conditional *Nedd4-2* deficiency (*Nedd4-2*$^{-/-}$) produces a IPF-like pulmonary disease. Our aim was to phenotype initial and progressive lung disease in *Nedd4-2*$^{-/-}$ mice by longitudinal *in vivo* micro-computed tomography (µCT).
Methods: *In vivo* cross-sectional and longitudinal µCT with a resolution of 35 µm was performed in conditional *Nedd4-2*$^{-/-}$ mice and littermate controls 1, 2, 3, 4 and 5 months after conditional *Nedd4-2* deletion. After *in vivo*imaging, mice were euthanized at each timepoint for *post mortem* µCT at 9 µm resolution. µCT images were evaluated for pulmonary fibrosis using an adapted fibrosis score.
Results: We were able to monitor disease progression of pulmonary fibrosis *in vivo* and quantify distinct radiological IPF key features along the disease development in conditional *Nedd4-2*$^{-/-}$ mice. Unspecific interstitial alterations were detectable even at early stages whereas IPF key features such as honeycombing-like lesions develop late in disease progression.
Conclusion: Longitudinal *in vivo* µCT imaging offers a sensitive, non-invasive method for monitoring IPF-like disease in conditional *Nedd4-2*$^{-/-}$ mice, providing new insights into pathogenesis and supporting preclinical evaluation of new diagnostic and therapeutic strategies.

Abstract No. 432

Behaviour and effects of nanoparticles on neutrophil recruitment in the pulmonary microcirculation

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Engineered nanoparticles hold great potential in precision drug delivery to the lung. However, interactions ("targeting") and potential adverse effects of NPs in the pulmonary microcirculation under healthy or pathophysiologic conditions are largely unknown.
To visualize and quantify NP dynamics and vascular pulmonary innate immune responses elicited by NPs in real-time, we applied intra-vital microscopy in the alveolar region of murine lungs. Two distinct NP subsets, exhibiting either a very low (PEG-amine-QDs), or a very high potential (carboxyl-QD) to interact with biomolecules and cells, have been used.
I.v. injected aPEG-QDs and carboxyl-QDs had a half-life time of 30 and 6 mins in the microcirculation and did not permanently interact with vessel wall. Blood flow velocity was significantly decreased after aPEG-QDs application in healthy mice, but did not further decrease the reduced microcirculatory perfusion after LPS treatment. In healthy mice, aPEG-QDs elicited a significant increase in neutrophil number in alveolar micro-vessels from 30 min upon application, whereas cQDs induced only a slight neutrophil increment. Accordingly, the crawling velocity of neutrophils in pulmonary microvessels was significantly reduced after aPEG-QDs application. No effect of QDs on neutrophil number and crawling was detected in the LPS groups. Importantly, neutrophil recruitment elicited by aPEG-QDs was diminished by prior i.v. application of anti-TNF-α, anti-E-selectin and anti-LFA-1 blocking antibodies.
Taken together our data demonstrates surface-dependent proinflammatory effects and reduced microcirculatory perfusion of QDs in healthy mice, whereas QDs application did not alter LPS-dependent microvascular inflammation.

Contribution of a Disease-spanning Working Group: Lung Environment Interaction

Abstract No. 433

Evidence for a lipofibroblast-to-Cthrc1^{pos} myofibroblast reversible switch during the development and resolution of lung fibrosis

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Genetic lineage tracing of alpha smooth muscle actin (Acta2) cells in the context of bleomycin-induced lung fibrosis in mice has shown that activated myofibroblasts (aMYFs) differentiate into lipofibroblasts (LIFs) during fibrosis resolution.
Acta2^{CreERT2}; Tomato^{flox} mice were used to label Acta2$^{positive(pos)}$ cells. Using scRNA-seq, the origin and fate of Acta2^{pos} cells converging on the aMYF/Cthrc1^{pos} myofibroblast lineage, were analyzed at the peak of fibrosis as well as during resolution.
Labeling of Acta2^{pos} cells before bleo-injury, displayed minimal contribution of these cells to the Cthrc1^{pos} pool. By contrast, labeling of cells following bleo-injury, led to massive contribution to Cthrc1^{pos} pool, which following fine clustering were grouped into 4 subclusters (Ct1-4). Interestingly, cells in Ct1 were identified as Cthrc1^{low} and displayed a strong LIF signature (LIF^{high}) while cells in Ct2 were LIF^{low} and Cthrc1^{high}. Examination of Ct1 and Ct2 subclusters during fibrosis resolution suggested that Ct2 transition back to a Ct1 status and eventually differentiate into Cthrc1^{neg} LIF^{high} alveolar fibroblasts. Analysis of the alveolar fibroblast cluster revealed the presence of three subclusters (Al1-3). Cluster Al3 appeared only when Acta2^{pos} cells were labeled following bleo-injury and displayed a fibrotic signature. Al3, which originate from Acta2^{neg} alveolar fibroblasts are suggested to differentiate into the Cthrc1^{low} LIF^{high} Ct1 subcluster. Data mining of human scRNA-seq from IPF lungs supported the conservation of the heterogeneity of the CTHRC1^{pos} population.
Our results support a LIF-to-aMYF reversible switch during fibrosis formation and resolution. Importantly, our work identifies a subcluster of aMYFs that is potentially relevant for future management of IPF.

Contribution of a Disease-spanning Working Group: Single Cell Analysis

Abstract No. 434

Uncovering the role of early virome in the development of moderate/severe bronchopulmonary dysplasia

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Bronchopulmonary dysplasia (BPD) in premature neonates has severe implications for pulmonary and extra-pulmonary long-term morbidity. Previous studies have identified characteristics in the airway bacteriome at birth in preterm neonates who developed BPD. Yet, the role of virome in these neonates remained to be explored.
Here, we characterized bacterial, viral, and fungal communities in samples from premature neonates, their mothers and the surrounding environment obtained from birth until day 28 of life using shotgun metagenomics.
Our multi-omics factor analysis showed that neonatal virome and bacteriome during the early postnatal period contributed significantly to the variation between the presence of no/mild and moderate/severe BPD than fungome did. In addition, differential abundance analysis identified three phages that were specifically abundant in neonates who developed moderate/severe BPD. Interestingly, we could identify these phages in their mother's milk and samples from the local surrounding, indicating the role of the environment in shaping the early virome, highlighting potential impacts on health. We detected a positive correlation between the abundance of these phages and their predicted host only in neonates who developed moderate and severe BPD. This suggests a potential role for the bacteria and its phages in disease severity.
Our study highlights the role of the early virome and bacteriome in lung disease development in preterm neonates and suggests the expansion of certain species as a potential biomarker for early diagnosis of the disease, which hold great potential in the prevention and management strategies.

Contribution of a Disease-spanning Working Group: Microbiome-Metagenome

Abstract No. 435

Antifibrotic potential of the Heparane Sulfate mimetics

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Idiopathic pulmonary fibrosis is a chronic interstitial lung disease. The therapy is very limited, as in other fibrotic diseases, and the current research needs alternatives. The presence of the myofibroblasts (MYF) is the most predominant feature in fibrotic diseases. MYFs respond to different signal molecules, e.g. TGFβ1, abnormally present in the microenvironment. MYFs are characterized by contractile muscle fibers and deposit an excessive amount of extracellular matrix (ECM), which lead to reduced contractility and disrupted architecture. Among ECM molecules, heparan sulfate proteoglycans (HS) have important physiological functions: HS bind different proteins increasing their stability and bioavailability. These targets include structural proteins, growth factors and cytokines. To investigate the impact of the HS mimetics (HSm) in fibrosis, we induced a MYF phenotype using rhTGFβ1 in a cell-based *in vitro* model, a fibroblast cell line, WI-38.

We tested HSm alone or with other drugs (e.g. SB431542, a TGFβ1 receptor inhibitor) in MYF-induced cells. We analyzed the results by functional and transcriptomic analysis. The preliminary results showed a reduction in MYF markers expression in the HSm treated cells indicating an antifibrotic effect, and a general change in ECM genes expressions. We observed a combinatorial effect with other drugs and the functional assays supported the idea of a HSm positive contribution in the proliferation of epithelial cells.

In conclusion, HSm has shown a potential anti-fibrotic effect, alone and in combination with other drugs and could represent an alternative and/or a reinforcement in the current treatment of the fibrotic diseases. However, further investigations are needed to test the safety and the possible translational application.

Abstract No. 436

"Lung aging in a dish" - Epithelial cellular senescence and pro-fibrotic changes in human precision-cut lung slices

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Hallmarks of aging like cellular senescence are increased in idiopathic pulmonary fibrosis (IPF). To study aging-related mechanisms of IPF pathogenesis, we used ionizing radiation (30Gy) to induce cellular senescence in human precision-cut lung slices (hPCLS) that preserve lung architecture and cellular composition. Viability was evaluated by WST-1 and LDH assays and apoptosis by cleaved caspase 3. Senescence and fibrosis markers were evaluated by RT-qPCR, flow cytometry, and immunofluorescence (IF), and the senescence-associated secretory phenotype (SASP) was profiled using cytokine arrays. Epithelial stem cell capacity was assessed in organoid assays. Bulk RNA-sequencing was used to further characterize irradiated (IR) hPCLS 7 days post irradiation (dpir). IR-hPCLS remained viable 7 dpir with no significant increase in LDH release or apoptosis. IF showed induction of DNA damage (γH2Ax), cell cycle arrest (p21), and Senescence-Associated-ß-Galactosidase (SA-ß-Gal) activity 7 dpir. Senescence was mainly induced in the epithelial compartment as showed by reduced progenitor capacity and increased SA-ß-Gal activity in EpCAM+ cells. Transcriptomics and longitudinal SASP profiling showed upregulation of pathways related to inflammation, DNA damage, epithelial reprogramming, and senescence and downregulation of cell cycle progression and apoptosis. Finally, fibrosis markers such as COL1A1, ACTA2, PAI1, and TGFB1 and the release of Collagen type III N-terminal propeptide were induced 7 dpir. In conclusion, we present a novel ex vivo model of epithelial cellular senescence and early fibrotic changes. Future work will focus on identifying novel SASP biomarkers and establishing a platform for testing senolytic compounds to reverse the observed senescence-mediated fibrotic changes

Contribution of a Disease-spanning Working Group: Single Cell Analysis

Abstract No. 437

Comprehensive Structure Activity Relationship Analysis of antifibrotic N23Ps using a MetaSAR Model

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Idiopathic Pulmonary fibrosis (IPF) is an interstitial lung disease that leads to chronic remodeling of the lung tissue as a result of progressive fibrosis. Patients with IPF have a median survival of 3-5 years post-diagnosis. Novel antifibrotic therapies are needed since the approved pharmacotherapies can only slow the progression of IPF and other progressive interstitial lung diseases. Through high content drug screening of approved drugs, Tranilast was found to induce an antifibrotic phenotype at high concentrations in IPF patient-derived lung fibroblasts (pHLFs). We employed an ECM deposition assay using established ECM markers to conduct structure-activity relationship (SAR) analysis for novel antifibrotics produced by medicinal chemistry based on Tranilast's chemical backbone. This resulted in the discovery of highly potent N23Ps (N-(2-butoxyphenyl)-3-(phenyl)acrylamides). Over 100 N23Ps with structural variations were further synthesized and tested for improved activity as well as metabolic stability. To streamline the analysis and gain maximum insights from the data, we developed a dose response data analysis pipeline, called MetaSAR, capable of outlier & edge-effects detection, quality control, toxicity estimation, and automated generation of dose-response curves. Using this model, dose response data from all tested compounds were compiled into a comprehensive N23P SAR database. The ECM deposition assay coupled with the developed MetaSAR model hence effectively facilitates SAR analyses and lead compound optimization in the antifibrotic drug development.

Abstract No. 438

Persistent tachypnea of infancy (NEHI). A condition underdiagnosed in adult pulmonary medicine?

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Persistent tachypnea of infancy (PTI) is a recently described rare paediatric interstitial lung disease. The disease is categorized under "specific conditions of undefined etiology" and sometimes labelled as neuroendocrine cell hyperplasia of infancy (NEHI) due to specific histopathological features. The diagnosis can be made by typical clinical presentation and characteristic chest high resolution computed tomography after exclusion of other causes. The natural course of the disease until school age is known and is in generally favourable. However, there are indications of residual disease burden beyond school age and into adulthood.
We identified 4 patients older than 10 years, of which at least two CT scans were available. Follow-up clinical data and radiological findings were evaluated.
Typical radiological findings of PTI are ground glass opacities (GGO) in characteristic distribution, i.e. paramediastinal, parahilar, middle lobe and lingual. These were identified even at older age. Patient history showed clinical symptoms consistent with PTI within the first year of life in all of the patients. 75% of the patients had usual GGO pattern upon first CT scan and showed persisting GGO in similar distribution after 6 to 10 years. In two patients, additional changes due to progressing lung disease were found. One patient showed an atypical initial CT with mosaic attenuation and no residual changes at follow-up. Pulmonary function tests were moderately restrictive in all. All patients had respiratory symptoms at the time of the second CT scan.
Not all children with PTI/NEHI may have a favourable disease course. Persistence of symptoms and imaging abnormalities need to be evaluated in the long term as some patients with NEHI may progress.

Abstract No. 439

Counter-Regulation of the Proteasome Activator PA200 and the Immunoproteasome in Lung Fibrobroblasts – Implications for Anti-viral Immune Responses in IPF

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The immunoproteasome (IP) is a specialized form of the proteasome, expressed mainly in immune cells, or upon stimulation with inflammatory cytokines. The three catalytic immunoproteasome subunits generate antigenic peptides for MHC class I presentation and improve responses to CD8+ T cell in anti-viral immunity. We have recently shown that immunoproteasomes are upregulated in aberrant epithelial cells in IPF lungs. Similarly, the proteasome activator PA200 is induced in fibroblast foci of fibrotically remodeled lungs.
We here aim to investigate whether the immunoproteasme and PA200 are co-regulated by profibrotic stimuli such as TGF-beta.
We demonstrate that PA200 is able to bind the immunoproteasome in a similar manner as the standard proteasome using cryo-EM structure analysis of purified human complexes. In primary human lung fibroblasts (phLF), however, we observed downregulation of immunoproteasome expression by TGF-beta while PA200 was induced, both on RNA and protein level in donor and IPF-derived phLF. This finding was confirmed using mass spectrometry analysis of crosslinked proteasome complexes: TGF-beta treatment of phLFs increased formation of PA200-containing proteasome complexes while incorporation of the three catalytic immunosubunits was clearly reduced. TGF-beta also counteracted interferon-induced upregulation of the immunoproteasome in phLF. These data suggest that TGF-beta signaling might result in impaired immunoproteasome-mediated generation of antigenic viral peptides and defective activation of anti-viral cytotoxic CD8+ T cells. We are currently investigating this hypothesis using CD8+ T cell assays an infection models.

Abstract No. 440

The role of the microRNA-15 precursor family in normal and aberrant late lung development.

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Rationale: Bronchopulmonary dysplasia (BPD) is a chronic lung disease associated with preterm birth, and characterized by disordered lung development, with affected lungs exhibiting fewer alveolar air sacs, thickened septa, and fewer blood vessels, which limit gas-exchange capacity. In this report, the potential role of microRNA (miR)-15 family members as possible regulators of gene expression that control both physiological and pathological processes in lung development was explored.

Methods: The impact of hyperoxia (85% O2) on microRNA expression in mouse pup lungs was evaluated by microarray. Stereological analysis was performed post-miRNA inhibitor treatment of mice to investigate the role of the miR-497-5p on lung morphology. MicroRNA expression in different lung cell populations was evaluated after fluorescence-activated cell sorting. Localization of the miR-497-5p was assessed by fluorescence *in situ* hybridization. Possible microRNA targets were predicted *in silico*. Batch RNA-Seq analysis was performed on treated primary cells and mice.

Results: miR-497-5p levels were reduced in lungs of pups exposed to hyperoxia, as revealed by microarray profiling and real-time RT-PCR analyses. Human BPD patient lungs displayed a similar pattern. The miR-497-5p expression under hyperoxia was localized to lung epithelial cells. Mouse pups, after treatment with a miR-497-5p inhibitor, phenocopied hyperoxia-exposed animals. *In silico* tools revealed members of the Wnt family as possible targets of miR-497-5p, which was confirmed by batch RNA-Seq.

Conclusions: The role of miR-497-5p in aberrant lung development is associated with clinical and experimental BPD.

Abstract No. 441

Effects of long-term oxygen therapy on p53 dependent cellular crosstalk in idiopathic pulmonary fibrosis- Studies in advanced organoid models.

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Rationale: There is insufficient research on the harmful effects of long-term oxygen therapy (LTOT) on pulmonary fibrosis, specifically whether moderate oxygen concentrations can cause gas-exchange area destruction via endothelial-to-mesenchymal transition (EndMT).
Objective: We will use an advanced cell culture model that combines alveolar organoids and endothelial cells (ECs) to recreate the active interface between the microvascular endothelium and the neighbouring alveolus with parenchymal tissue and delineate the effect of supplemental oxygen focusing on p53 pathway signalling as a driver of EndMT.
Methods: EC injury will be initiated *in vitro* through exposure to moderate hyperoxia (FiO_2=0.4) or room air (FiO_2=0.21) in a time-dependent manner. Characterization for EC and fibroblast marker expression, apoptosis, proliferation, p53 expression, cell cycle regulation, and functional capacities will be complemented by secretome analysis. To facilitate investigation of the alveolar barrier for endothelial-epithelial crosstalk, hPSC-derived alveolar organoids will be grafted onto a chip-based, perfusable vascular bed.
Results: A decrease in functionality of hyperoxia exposed ECs was observed, evident by reduced tube formation, decrease in expression of EC markers, increase in expression of mesenchymal and inflammatory markers; p53 downregulation as a critical regulator of cell cycle progression. We plan to conduct further studies using alveolar organoids implanted on a chip-based, perfusable vascular bed.
Conclusion: The results highlight importance of understanding molecular mechanisms underlying EC dysfunction under oxygen therapy, transition from an endothelial to a mesenchymal cell phenotype may be a key player in detrimental effects when treating IPF patients with LTOT.

Abstract No. 442

Prolyl-3-hydroxylase 4 (P3H4), a novel player in collagen synthesis and secretion in lung fibroblasts

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Idiopathic pulmonary fibrosis (IPF) is characterized by fibroblast activation and accumulation of collagen in the alveolar space. We have previously proposed the collagen chaperone FK506-binding protein 10 (FKBP10) as a therapeutic target for IPF. Expression of *FKBP10* and prolyl-3-hydroxylase 4 (*P3H4*), a novel component of the ER-resident collagen folding complex, are controlled by a common bidirectional promoter, but regulation and function of P3H4 in lung fibrosis is unknown. Transcriptomic and proteomic analysis was performed using lungs from FKBP10 knockout mice. Functional analysis was performed using siRNA-mediated knockdown in an *in vitro* model of lung fibrosis where primary human lung fibroblasts (phLF) are treated with transforming growth factor-β1 (TGF-β1). Expression of FKBP10, P3H4, profibrotic markers, and total collagen secretion was assessed using qRT-PCR, immunoblotting, and Sircol assay. Protein-protein interactions were characterized with proximity ligation assay (PLA) and immunofluorescence (IF). Transcriptomic and proteomic analysis of FKBP10 knockout mouse lungs revealed downregulation of P3H4. Similarly, knockdown of FKBP10 resulted in downregulation of P3H4 in phLF and *vice versa.* As previously reported for FKBP10, knockdown of P3H4 in phLF decreased synthesis and secretion of collagen. PLA and IF supported colocalization of FKBP10 and P3H4 in the ER of phLF as well as in fibrotic tissue sections from bleomycin-treated mice and IPF patients. P3H4 expression was upregulated by TGF-β1 in phLF and in IPF lung extracts. In conclusion, P3H4 and FKBP10 reciprocally regulate each other in lung fibroblasts, and P3H4 represents a novel modulator of collagen biosynthesis in the lung and hence a potential therapeutic target for IPF.

Abstract No. 443

Sex and gender-specific aspects associated with undiagnosed interstitial lung disease in patients presenting for bronchoscopy with transbronchial cryobiopsy at a Specialised ILD centre

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Introduction
Gender-specific aspects are established in idiopathic pulmonary fibrosis, but unknown for undiagnosed ILD
Methods
Consecutive patients with ILD who underwent bronchoscopy with TCB and if necessary surgical lung biopsy (SLB) 11/17-12/21 at Thoraxklinik Heidelberg were analysed. In addition to medical history and functional diagnostics, CT biomarkers, visual and via fully automated segmentation of the 80th and 40th percentile of lung density, reflecting fibrosis and ground-glass opacity (GGO) as well as the emphysema index (%) were examined using the YACTA software tool. The diagnoses according to multidisciplinary ILD board discussion (MDD) were analyzed with regard to sex and gender differences.
Results
Data from a total of 404 patients aged 67.8 ±11.8 years, 155 (38%) female, FVC 83,9% pred. and DLCO 56.5% pred. were analyzed. We found no sex differences with respect to age, pulmonary function, or comorbidities. With a proportion of 46.5% never smokers and 53.5% active or former smokers with 30.5±21.8 py, there were no differences in smoking behaviour between the sexes. Imaging also revealed no differences between the sexes in visual assessment of indeterminate UIP or NSIP patterns, reticular patterns, and GGO and the 80th and 40th percentiles. After TCB or SLB (7,2%), a diagnosis according to MDD was made in >90 % of cases. The most common diagnoses were HP, sarcoidosis, IPF, NSIP, and unclassifiable ILD, with no gender differences in the frequency of each entity.
Conclusion
In the analysis of a real life collective of different ILD, no gender-specific differences were found with regard to radiological patterns, severity of disease and diagnosis according to MDD. The careful differential diagnostic workup should be performed in a gender-neutral manner.

Abstract No. 444

Identification of antifibrotic N23Ps' mode of action by advanced human fibrosis models

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Idiopathic pulmonary fibrosis is a fatal lung disease characterized by the scarring of parenchymal and interstitial tissue due to fibroblast activation and excess ECM deposition mostly TGFβ1 driven. Current pharmacotherapies only decelerate course of disease, highlighting the unmet need for novel therapies. Gerckens et al. developed a phenotypic high-throughput drug discovery screening assay (HT-A) applied to the Prestwick Clinical Library that identified Tranilast an N2CAB (N-(3',4'-dimethoxycinnamoyl)-anthranilic-acid) as an ECM deposition inhibitor. Quantitative structure activity relationship (SAR) studies of Tranilast derivates led to the discovery of N-(2-butoxyphenyl)-3-(phenyl)acrylamides (N23Ps), a novel class of antifibrotic compounds featuring higher potency in fibroblasts. We aim to characterize N23Ps' mode of action (MoA) in respect to myofibroblast differentiation, cell morphology alterations, and gene expression profile in 3D lung tissue microenvironment. Preliminary bioinformatic analysis of omics data derived from fibrosis models: *in-vitro* TGFβ1+N23P treated primary human lung fibroblasts (phLFs) and *ex-vivo* fibrotic cocktail-N23P-treated precision cut lung slices (hPCLS), indicated a deregulation of cytoskeletal networks. We aim to validate this hypothesis by establishment of a morphology fingerprint using different morphology parameters processed via deep learning, dimensionality reduction, and treatment clustering of various patients. Ongoing validation experiments aim to further support a cytoskeletal-based MoA. Additional proteomic analysis of N23Ps-treated phLFs and hPCLS assessed via scRNAseq could set the base for the identification of novel drug effects, a MoA that differs from N2CABs, and a similar or better clinical outcome compared to N2CABs

Contribution of a Disease-spanning Working Group: Single Cell Analysis

Abstract No. 445

Impact of GPRC5A on myofibroblasts differentiation and fibrosis

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Rationale: the G protein-coupled receptor, class C, group 5, member A (GPRC5A), whose ligands are largely unknown and whose downstream pathway is not fully understood, is mainly studied in different human cancers and described as a tumor suppressor gene, downregulated in non-small cell lung carcinoma. New evidence suggests that GPRC5A plays a key role in the pathogenesis of lung fibrosis. *Gprc5a* knock-out mice have a higher susceptibility to fibrosis compared to the wildtype. Metadata analysis of human lung samples shows a downregulation of *GPRC5A* in idiopathic pulmonary fibrosis (IPF) .We investigated the effect of *GPRC5A* on fibroblasts *in vitro*, focusing on their shift between myofibroblasts (MYF) and lipofibroblasts (LIF).
Methods: we analyzed GPRC5A *expression* in lung samples of IPF and healthy donors, primary fibroblast and WI-38 cell cultures. We treated fibroblasts *in vitro* with TGFβ1 or metformin (MET) to differentiate into the MYF or LIF phenotype and analyzed the level of expression of *GPRC5A*. Using overexpression or knockdown of *GPRC5A,* we tested whether we could in turn induce a shift towards a specific phenotype.
Results: we confirmed the downregulation of *GPRC5A* in lung samples of IPF patients compared to healthy donors. MYF differentiation decreased *GPRC5A* expression, while it increased in MET differentiated LIF. The knockdown of *GPRC5A* resulted in upregulation of *MYF markers* indicating a shift towards the MYF phenotype. If *GPRC5A* is overexpressed in MYF *ACTA2* expression is reduced compared to controls, indicating a key role of *GPRC5A* in the fibroblast differentiation.
Conclusion: the downregulation of *GPRC5A* is characteristic of myofibroblasts and necessary for their differentiation.

Abstract No. 446

Defining circulating myeloid skewing in Idiopathic pulmonary fibrosis

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Idiopathic pulmonary fibrosis (IPF) is a challenging condition with limited treatment options. Monocytes have been identified as potential biomarkers for IPF progression, but which specific myeloid subtypes are involved is unclear.
In this study, we used multi-color flow cytometry to analyze circulating myeloid subsets in the blood of IPF patients. Compared to the control, we found increased immature precursors of monocytes and classical monocytes. Using single-cell transcriptomics in magnetically-purified monocytes, we identified several differentially expressed genes and 7 distinctive monocyte clusters. Of the 7 clusters, some were previously related to kidney fibrosis development and others to COVID-19 transition from severe disease into full fibrosis. We investigated the role of immature precursors of monocytes in autologous co-cultures with T cells. We found that immature monocytes decreased T cell proliferation, induced de novo exhaustion, and formation of regulatory T cells. We performed a three-dimensional collagen invasion assay showing that immature monocytes in IPF patients have a higher invasive potential than the control.
Taken together, immature suppressive monocytic subsets are expanded in the peripheral blood of IPF patients and might induce an immunosuppressive environment. Further research is needed to understand their role and potential as therapeutic targets.

Contribution of a Disease-spanning Working Group: Single Cell Analysis

Abstract No. 447

Genetic Deletion of *Muc5b* in Pulmonary Epithelial Cells Ameliorates Childhood Interstitial Lung Disease in Congenital *Nedd4-2* Deficient Mice

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Several studies have shown that overexpression of the mucin MUC5B is one of the major risk factors for the development of idiopathic pulmonary fibrosis in adults. However, little is known about the role of MUC5B in interstitial lung disease in children (chILD). By congenital deletion of *Nedd4-2* (*Nedd4-2*$^{-/-}$) in murine lung epithelial cells, we recently generated a new mouse model sharing clinical and histological key features with chILD. By genetic deletion of *Muc5b* in congenital *Nedd4-2*$^{-/-}$ (*Nedd4-2*$^{-/-}$/*Muc5b*$^{-/-}$) mice, we intend to investigate the role of Muc5b in the pathogenesis of chILD.

To determine the effect of *Muc5b* in chILD, we compared the clinical phenotype of congenital *Nedd4-2*$^{-/-}$ mice with congenital *Muc5b* deficient (*Muc5b*$^{-/-}$), *Nedd4-2*$^{-/-}$/*Muc5b*$^{-/-}$ double-knockout mice and littermate controls at neonatal ages. Therefore, we assessed oxygen saturation, survival, inflammation in bronchoalveolar lavage (BAL) and lung morphology by hematoxylin and eosin and Alcian blue and periodic acid-Schiff staining.

Muc5b deletion in congenital *Nedd4-2*$^{-/-}$ mice resulted in an increased oxygen saturation and a reduced mortality five weeks after birth compared to congenital *Nedd4-2* deletion alone. We further found less inflammatory cells in BAL and milder pneumonitis in histology in *Nedd4-2*$^{-/-}$/*Muc5b*$^{-/-}$ double-knockout mice compared with *Nedd4-2*$^{-/-}$ mice.

Taken together *Muc5b* deletion in lung epithelial cells ameliorates chILD in congenital *Nedd4-2*$^{-/-}$ mice and may serve for targeted therapeutic strategies in the treatment of chILD.

Abstract No. 448

GR-Scale questionnaire mirrors functional impairment in various ILD diagnoses

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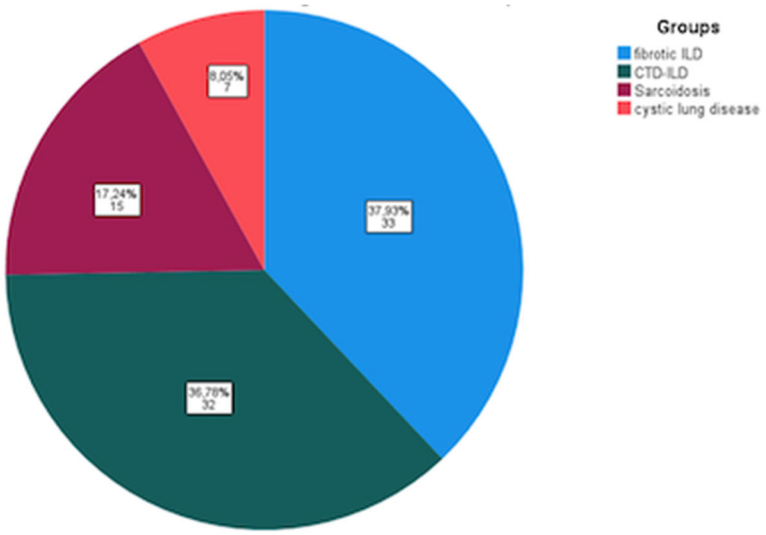
Background
Scallan et al. [1] proposed the R-Scale as a new questionnaire to assess the health status in patients with IPF.

Objective
We aimed to investigate the utility of a German version of the R-Scale (GR-Scale) by analyzing its relationship to lung function parameters in various ILD at baseline.

Methods
87 patients from four different diagnostic ILD groups (see figure) completed the GR-Scale questionnaire. Pearson correlation coefficients were calculated between GR-Scale scores and routine lung function parameters (forced vital capacity (FVC) and diffusion capacity for carbon monoxide (DLco)).

Results
Within the entire study population, inverse correlations were found between the GR-Scale total score and FVC (r=-0.279 p=0.009) and DLco (r=-0.216 p=0.045), respectively. In group 1 (fibrotic ILD), similar negative trends existed for FVC (r=-0.33 p=0.062) and DLco (r=-0.31 p=0.075). For groups 2 (CTD-ILD) and 3 (Sarcoidosis) trends towards a negative correlation were found for FVC and DLco (not significant). In group 4 (cystic lung disease) FVC and the GR-Scale total score showed a positive trend (r=0.54 p=0.21).

Conclusion
Our study shows that the GR-Scale and routine lung function parameters show highly variable relationships, but also some consistent trends, thus possibly providing an additional dimension of information about the patients' health for clinical assessment.



Pie chart of the 4 groups (entire study population n=87)
Group 1 (n=33): fibrotic ILD (IPF, PPF cHP, NSIP, uILD)
Group 2 (n=32): CTD-ILD
Group 3 (n=15): Sarcoidosis
Group 4 (n=7): cystic lung disease (BHDS, CPFE, LAM, LIP)

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Abstract No. 449

NLRP3 inflammasome activation triggered by a disrupted cholesterol metabolism in alveolar macrophages of aging SP-C deficient mice

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Surfactant protein C (SP-C) is a hydrophobic protein found in lung surfactant (LS), responsible for the stability, lipid adsorption and arrangement of the LS membranes at the alveolar interface. SP-C absence in mice has been related to impaired cholesterol metabolism and accumulation of crystals in alveolar macrophages (AMs) [1]. Cholesterol content (5-10 % wt.) in LS is conserved for a proper surfactant function. AMs are involved in cholesterol homeostasis and previous studies proposed SP-C as a cholesterol metabolism regulator [2]. We hypothesize that these crystals are made of cholesterol and they activate NLRP3 inflammasome in AMs of SP-C deficient aging mice. To do so, expression of proteins involved in cholesterol metabolism and NLRP3 inflammasome were evaluated in 10, 30 and 60 weeks-old SP-C deficient mouse AMs using western blot. Total cholesterol in AMs and bronchoalveolar lavage fluid (BALF) was quantified by fluorimetric assay and IL18 levels in BALF were analysed by ELISA. Major expression of NLRP3 inflammasome proteins in the 60 week-old group was detected, together with higher cholesterol and IL18 levels. Additionally, transmission electron microscopy revealed electron-dense crystals, similar to cholesterol clefts in 60 week-old mouse AMs. These data suggest a NLRP3 inflammasome activation triggered by a disrupted cholesterol metabolism in 60 weeks-old AMs of SP-C deficient mice.

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Abstract No. 450

An *in vitro* model to study myofibroblast and lipofibroblast differentiation

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The majority of in vitro studies for myofibroblast/Lipofibroblast (MYF/LIF) differentiation are based on human primary fibroblast cultures (HPFc) derived from IPF patients. High variability among HPFc, availability and the limited time in culture represent major limitations. MYF or LYF differentiation has been shown in HPFc models upon treatment with TGFB1 or Metformin (Met)[1], respectively. The WI-38 cell line is a human embryonic fibroblast cell line that has been shown to differentiate to MYF when treated with TGFB1[2].
The aim of this work is to investigate the suitability of the WI-38 cells as a valid alternative to the HPFc to study MYF and LIF differentiation.
Treatment of WI-38 cells with TGFB1 or Met was used to induce MYF and LIF phenotypes, respectively. RNA-seq was performed on WI-38 cells and compared with published datasets of HPFc. Furthermore, alveolosphere, cell proliferation and wound healing assay were carried out. Comparison analysis confirmed the validity of the WI-38 cells as an in vitro model to study MYF and LIF differentiation, showed by the overlap in the MYF/LIF signatures from the HPFc and fresh lung tissue versus our model. Alveolospheres from AT2 cells and LIF showed an increase in the size. On another hand, MYF showed a significant decrease in the capability of wound closure. Consequently, WI-38 cell line is a good model to study MYF-LIF differentiation *in vitro*. The plasticity of these cells lends itself to be manipulated and studied in the fibrosis field.

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Abstract No. 451

The emergence of myeloid-derived suppressor cells in a murine model of pulmonary fibrosis

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Background: The immune system plays a major role in pulmonary fibrosis. Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of innate immunity with remarkable immune suppressive functions. We hypothesize that the strong anti-inflammatory activity of MDSCs may dampen pulmonary fibrosis by inhibiting local inflammation and its transition to lung fibrosis. *Aim:* Here, we aim to investigate the emergence of both polymorphonuclear (PMN)- and monocytic (M)-MDSCs and their pattern of generation, activity and recruitment in pulmonary fibrosis. *Method:* Murine model was performed by administration of bleomycin in C57BL/6 mice. Immunological, histopathological and clinical changes of mice respiratory tract were investigated at days 3, 7, 14, and 21 after bleomycin challenge. *Results:* Strikingly, we found entirely different pattern of PMN- and M-MDSCs generation and recruitment after bleomycin challenge. The number of PMN-MDSCs was increased on day 3 reaching a maximum on day 21 after bleomycin challenge. However, while the number of M-MDSCs were higher on day 3, their numbers decreased over time reaching to the minimum on day 21. The suppressive activity of PMN-MDSCs was mainly pronounced on day 3, but M-MDSCs retained their suppressive activity from day 3 to day 21. The changes in the number and activity of MDSCs were also correlated with lung histopathological and respiratory mechanics changes. *Conclusion:* We showed that the number and activity of PMN- and M-MDSCs are different from each in acute inflammatory and fibrotic stages. These findings suggest that MDSCs are involved in modulating the immunological, histopathological and functional changes in pulmonary fibrosis.
Keywords: Myeloid-derived suppressor cells, Animal model, Pulmonary fibrosis

Abstract No. 452

Persistent macrophage depletion and arrested replenishment is dependent on carbon nanotube type as shown by single cell transcriptomics

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Inhalation of carbon nanomaterials (NMs) can cause severe health effects, including chronic inflammation and fibrosis. We aim for a better understanding of NM specific cellular perturbation patterns. Therefore, we exposed mice intratracheally to soot like carbon black, tangled double-walled (DWCNT) and rigid, multi-walled carbon nanotubes (MWCNT). All lungs underwent single cell RNA sequencing and BAL analysis at 12h, 6d and 28d.
At the chosen doses, all NMs caused comparable airspace neutrophilia at 12h, which increased until d6 for CNTs and remained elevated until d28 for MWCNT.
We observed a significant increase in total BAL macrophages, however, exclusively after 6d CNT instillation. This d6 peak coincided with the loss of F4/80$^+$/CD11c$^+$/CD11b$^-$ alveolar macrophages (AMs) for MWCNTs, and to a lesser extent DWCNTs, in lung FACS.
In parallel, we noticed the pulmonary accumulation of a transitional macrophage population at d6 uniquely after MWCNT instillation, and not DWCNTs. According to RNA velocity analysis, blood monocytes differentiate into Lyve1$^-$/CD163$^-$ interstitial macrophages and subsequently give rise to this transitional population which fails to replenish the CD11c$^+$/SiglecF^{high} AM pool. These cells show high similarity with the initiating monocyte-derived macrophage population necessary for bleomycin- and asbestos-induced lung fibrosis (Misharin et al.,JEM,2017.214(8)). The pro-fibrotic role of this population also has been demonstrated in context of SARsCoV2 infection-associated acute respiratory distress (Wendisch et al.,Cell,2021.184(26)).
We summarize, that this transitional cell population are monocyte-derived AM precursors, which lack the ability to differentiate into mature AMs being the decisive mechanism for MWCNT induced, not resolving lung fibrosis.

Contribution of a Disease-spanning Working Group: Single Cell Analysis

Abstract No. 453

Co-cultured, human alveolar organoids recapitulate epithelial-mesenchymal crosstalk in fibrotic lung disease

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Interstitial lung disease (ILD) summarizes lung pathologies with destruction and incomplete regeneration of lung tissue, resulting in persistent architectural distortion. We seek to understand ILD-related mesenchymal-epithelial crosstalk and bridge the gap to identifying potential therapeutic strategies. To achieve this, we developed a co-culture system of human stem cell-derived alveolar organoids and ILD-patient fibroblasts in comparison to control fibroblasts. To identify secreted ligands associated with disease, supernatants from ILD and non-CLD fibroblasts were analyzed by mass spectrometry.
While organoid formation was decreased in the presence of lung fibroblasts independent of disease, size and morphology remained unchanged. Interestingly, metabolic activity was significantly increased in ILD co-cultures and stem cell markers such as Surfactant Protein C and Keratin 8 showed reduced transcription. Strikingly, an aberrant basaloid-prone expression profile was induced in ILD co-cultured organoids, indicated by elevated expression of Cadherin 2, Bone Morphogenic Protein 4, and Vimentin, all associated with aberrant epithelial differentiation. The secretome of ILD fibroblasts included pro-inflammatory cytokines and mediators of extracellular matrix remodeling with interleukins emerging as key mediators to guide mesenchymal-derived crosstalk in disease. Treating organoids with interleukins for functional validation recapitulated effects observed in ILD co-cultures. In summary, we identified mediators likely contributing to pathologic mesenchymal-to-epithelial crosstalk and confirmed key player in ILD pathogenesis. The novel organoid co-culture model provided functional evidence and enabled us to delineate fibroblast-initiated aberrant epithelial differentiation.

Abstract No. 454

Novel antifibrotic drug development through optimization of N23Ps and identification of molecular targets

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Lung fibrosis is a fatal interstitial lung disease with no known cure other than lung transplantation. Novel antifibrotic drugs are therefore urgently needed. In this study, we used a human fibrosis model based on ECM deposition of patient-derived IPF fibroblasts to develop N-(2-butoxyphenyl)-3-(phenyl)acrylamides (N23Ps) as a potential lead compound and to identify its molecular target. Over 100 derivatives of N23Ps were synthesized by medicinal chemistry and their efficacy was tested in a phenotypic assay by assessing their inhibition of aberrant extracellular matrix (ECM) protein deposition. We determined the structure-activity relationships (SAR) and comprehensively explored N23Ps' chemical space, identifying various novel and distinct modifications that exhibited similar ECM inhibiting activities as the original N23Ps. Additionally, we found modifications which improved the N23Ps' in-vitro serum stability and microsomal stability without reducing its potency, thus making it a possible candidate for in vivo efficacy testing. Furthermore, we also developed an active diazirine derivate that inhibits TGFβ1-induced ECM deposition. This compound allows for UV-photo-crosslinking to a target protein and a click-chemistry reaction to biotin in order to perform a chemical pull down to identify direct interaction partners of N23Ps. This will enable the identification of molecular targets and mechanisms of action of N23Ps. In conclusion, our findings provide a comprehensive exploration of N23Ps' chemical space, optimize their metabolic stability, and present active compounds for molecular target identification in the development of novel antifibrotic drugs.

Abstract No. 455

Modulation of LRP1, a lipid metabolism-related molecule, reverts profibrotic changes in lungs

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Idiopathic pulmonary fibrosis (IPF) is a severe disease of unknown etiology. Accumulating evidence suggests a multifactorial IPF mechanism, with alveolar epithelial injury, disturbed hemostasis, imbalanced lipid metabolism, and excessive extracellular matrix (ECM) deposition being involved. Considering that low-density lipoprotein receptor-related protein 1 (LRP1) is a major regulator of lipid metabolism and ECM turnover, we hypostatized its role in maladaptive lung tissue remodeling. We found decreased expression of LRP1 in lung fibroblasts (LF) isolated from IPF patients and bleomycin-treated mice. Knockout of *Lrp1* in PDGFR-α^+ cells aggravated bleomycin-induced lung fibrosis and increased lipid accumulation in fibrotic lung regions. Likewise, lipid accumulation was observed in IPF LF, following *LRP1* silencing in LF isolated from donors, and *Lrp1*-/- mouse embryonic fibroblasts (*Lrp1*$^{-/-}$MEF). Transcriptome analysis of *Lrp1*$^{-/-}$MEF revealed differential expression of multiple lipid-related genes, including those involved in Wnt signaling, angiogenesis, ECM-receptor interaction, and ECM organization. Lipidome analysis of *Lrp1-/-*MEF showed deregulation of triglycerides and cholesterol esters. Finally, elevated levels of *LRP1*-natural antisense transcript *LRP1-NAT* or its murine homolog were associated with reduced *LRP1* or *Lrp1* expression in lungs of IPF patients or bleomycin-treated mice, respectively. Depletion of *LRP1-NAT* in LF or IPF precision cut lung slices induced restoration of LRP1 levels and reversal of a profibrotic cell/tissue phenotype. Altogether, our results highlight pivotal role of lipids in the regulation of fibrotic processes and provide evidence for therapeutic potential of lipidome manipulations in the treatment of lung fibrosis.

Abstract No. 456

ABCA3 deficiency - variant-specific response to Hydroxychloroquine

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Background

Biallelic variants in *ABCA3*, the gene encoding the lipid transporter ATP-binding cassette subfamily A member 3 (ABCA3) and predominantly expressed in alveolar type II cells, may cause interstitial lung diseases in children (chILD) and adults. Currently there is no proven therapy, but hydroxychloroquine (HCQ) is frequently used empirically. We hypothesized that *in vitro* responsiveness to HCQ might correlate to patients' clinical outcome with HCQ therapy.

Methods

Clinical data of the subjects with chILD due to ABCA3 deficiency and treated with HCQ were retrieved from the literature and the Kids Lung Register data base. *In vitro* experiments were conducted on wild type (WT) and 16 mutant ABCA3-HA transfected A549 cells. Responses of the functional read out were assessed as the extent of deviation from untreated WT.

Results

With HCQ treatment, 19 patients had improved or unchanged respiratory condition, and 20 had respiratory deteriorations, 5 of whom transiently improved then deteriorated. *In vitro* ABCA3 functional assays identified 2 variants with complete response, 5 with partial response and 9 with no response to HCQ. Variant-specific HCQ effects *in vivo* closely correlated to i*n vitro* data. $ABCA3^+$ vesicle volume above 60% of WT volume was linked to responsiveness to HCQ; HCQ treatment response was concentration dependent and differed for variants *in vitro*.

Conclusions

We generated evidence for an *ABCA3* variant-dependent impact of HCQ *in vitro*. This may also apply for HCQ treatment *in vivo*, as supported by the retrospective and uncontrolled data from treatment of chILD due to ABCA3 deficiency.

Abstract No. 457

Role of lipofibroblasts in the development and resolution of pulmonary fibrosis

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Idiopathic pulmonary fibrosis (IPF) is an extremely aggressive and fatal form of interstitial lung disease (ILD) and current antifibrotic therapies only slightly alleviate disease progression. Lung fibrosis is associated with repetitive injury to alveolar epithelial cells (AECs), recruitment of inflammatory cells, profibrotic cytokine signaling and aberrant repair responses that ultimately lead to respiratory failure. The precise cellular source and ultimate fate of myofibroblasts, which are the effector cells in IPF, have been a controversial topic. Fortunately, recent advances in lineage-tracing models have helped address lung myofibroblast precursors and revealed additional contributors to the heterogenous myofibroblast population. Identifying the primary sources of myofibroblasts could be a promising strategy to develop novel anti-fibrotic therapies. It has been previously shown that lipofibroblasts transdifferentiate into myofibroblasts during the development of pulmonary fibrosis. Such myofibroblasts can undergo apoptosis or redifferentiation into lipofibroblast-like cells during fibrosis resolution. Studying this phenomenon offers a promising prospect to identify signalling pathways that lead to accumulation of lipofibroblast-like cells and re-engagement of these cells to promote lung repair. In this work we used state-of-the-art techniques including lineage tracing, single-cell RNA sequencing (scRNA-seq) and alveolar organoids to characterize lipofibroblasts and lipofibroblast-derived cells during various phases of fibrosis formation and resolution. Our preliminary data highlight the heterogeneity of lipofibroblasts and their descendants during fibrosis formation and resolution, and shed light on the impaired alveolar niche activity in the fibrotic lung.

DA Pulmonary Hypertension: Abstract No. 501 – 531

Abstract No. 501

GLI1+ cells contribute to muscularization in hypoxia and cigarette smoke-induced pulmonary hypertension

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Pulmonary hypertension (PH) is largely present in patients suffering from COPD. The precise origin of newly formed ACTA2+ cells appearing in initially non-muscularized vessels in the context of pulmonary hypertension (PH) induced by chronic hypoxia (HOX) or cigarette smoke exposure (SE) is still debated. . In this work, *Gli1*$^{Cre\text{-}ERT2}$*; tdTomato*^{flox} mice were used to lineage-trace GLI1+ cells in lungs exposed to hypoxia (HOX) versus normoxia (NOX) or cigarette smoke (SE condition) versus room air (RA). Lungs were also analyzed during PH reversal following re-exposure to NOX or RA alongside with the treatment of L-NIL, respectively. Hemodynamic measurements, right ventricular hypertrophy assessment, single-cell RNA sequencing (scRNA-seq) on FACS-sorted GLI1+ cells, immunofluorescence (IF) on precision-cut lung slices (PCLS) and IMARIS 3D quantification were carried out. *Gli1*$^{Cre\text{-}ERT2}$; *tdTomato*^{flox}*; iDTR*^{flox} mice were additionally generated to ablate GLI1+ cells. Analysis of lineage-traced GLI1+ cells by FACS and 3D imaging indicated that GLI1+ cells contribute to both muscularization of already muscularized proximal vessels and neomuscularization of distal vessels. Upon PH reversal, GLI1+ cells are cleared from the distal vascular compartment. scRNA-seq on GLI1+ cells confirmed the presence of an *Acta2*^{high} fraction, and trajectory analysis suggested that ACTA2+ GLI1+ cells are derived from an existing pool of progenitors within this cluster. Genetic ablation of GLI1+ cells was protective against hypoxia-induced PH in mice. Finally, modulation of the hedgehog/GLI1 axis in pulmonary arterial smooth muscle cells and adventitial fibroblasts derived from donor and idiopathic pulmonary arterial hypertension (IPAH) patients showed that targeting this pathway has promising effects.

Abstract No. 502

AK4-mediated hyper-proliferation in pulmonary vascular cells as a therapeutic implication in pulmonary hypertension?

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Pulmonary hypertension (PH) is a life-threatening disease, characterised by a mean pulmonary artery pressure (mPAP) ≥ 20 mmHg at rest. Alterations in the pulmonary vasculature, such as hyper-proliferation of pulmonary arterial smooth muscle cells (PASMC), endothelial cells and fibroblasts, lead to vessel lumen obliteration, resulting in elevated vascular resistance and the development of PH. One of the molecular mechanisms driving PH development is the hypoxia-inducible factor 1-alpha (HIF1-α)-dependent metabolic shift in pulmonary vascular cells. Previously, we identified the adenylate kinase 4 (AK4) as a hypoxia/HIF1-α-regulated gene and a possible regulator of the HIF1-α-dependent glycolytic shift in hypoxic PASMC. Furthermore, we detected AK4 upregulation in hypoxic pulmonary microvascular endothelial cells (PMVEC). However, the molecular mechanisms underlying AK4-mediated regulation of HIF1-α and the role of AK4 in the pathogenesis of PH remain unresolved.

Against this background, we investigated kinome profiles upon AK4 loss-of-function using small interfering RNA (siRNA) in long-term hypoxic air (1% O_2)-exposed PASMC and PMVEC. The analysis revealed that AK4 silencing in hypoxic PASMC and PMVEC altered activities of several kinases (e.g. Src family kinases (SFK), cyclin-dependent kinases (CDKs) and mitogen-activated protein kinases (MAPKs)), suggesting the pro-proliferative role of AK4 in pulmonary vascular cells. Further study will focus on investigating the *in vivo* function of *Ak4*-ablation in PASMC and endothelial cells, sought to provide an insight into a novel therapeutic strategy for PH.

In conclusion we suggest, that the potential AK4-HIF1-α feedforward loop promotes the pro-proliferative phenotype of vascular cells and thus contributes to PH development.

Abstract No. 503

Deciphering the spatial patterning and lung-specific pericyte marker code

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Pericytes are a unique population of mesenchymal cells embedded in basement membrane functioning as an interface between endothelium and perivascular niche. Several chronic lung diseases are linked with underlying perturbations in pericyte abundance and function. Unlike for other lung mesenchymal cells investigated at single cell resolution, little is known about the intrinsic pericyte heterogeneity in health and disease.
We performed a high resolution multispectral imaging of formalin-fixed paraffin-embedded human lung tissue from healthy controls, interstitial lung disease and chronic obstructive lung disease with pulmonary hypertension patients. Using a combination of in situ hybridization and immunofluorescence staining, we investigated the co-expression and localization of 7 canonical pericyte markers. Subendothelial expression localization could be detected for all tested markers except FABP4. Vascular-restricted expression in combination with variable overlap with ACTA2 (αSMA) expression was observed for NOTCH3 and CSPG4 (NG2). RGS5 had extensive overlap with vascular ACTA2 (αSMA) expression, but also displayed a promiscuous expression in other cell types, such as inflammatory cells. Similarly, COX4I2 and NDFUAL2 showed a patchy vascular ACTA2-overlaping expression pattern and positive signal in other cell types. PDGFRB showed the most prominent expression in the perivascular, adventitial region. Importantly, vascular-associated marker expression was preserved in the case of both chronic lung diseases.
In summary, we provide a topographic map of pericyte-linked marker expression at different anatomical levels in healthy and remodeled human lungs. The results will guide future studies to delineate the role of pericyte heterogeneity in chronic lung diseases.

Abstract No. 504

Mitochondrial Cytochrome *c* Oxidase Subunit 4 Isoform 2 (Cox4i2) Plays a Key Role in the Hypoxia-induced Reduction of the Electron Transfer System

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Introduction: Mitochondrial cytochrome *c* oxidase subunit 4 isoform 2 (Cox4i2) is essential for oxygen sensing in pulmonary arterial smooth muscle cells (PASMCs). Cox4i2-dependent superoxide production during acute hypoxia (AH) triggers the calcium signal to induce hypoxic pulmonary vasoconstriction, avoiding life-threatening hypoxemia. Nonetheless, the molecular mechanism by which Cox4i2 sensitizes the whole cytochrome c oxidase (COX) to sense oxygen remains unclear.
Methods: We analysed oxygen consumption by high-resolution respirometry, complexome profiling by blue native electrophoresis and mass-spectrometry, and redox changes of the electron transport system (ETS) by Raman spectroscopy of PASMCs isolated from wild type (WT) and Cox4i2$^{-/-}$ mice. To figure out the role of Cox4i2-specific cysteine residues we generated mouse epithelial (CMT167) cells overexpressing either Cox4i1, or WT Cox4i2, or Cox4i2 mutants (C41S, C55A, C109S), and we tested superoxide production and oxygen affinity.
Results: Respiration, abundance and assembly of COX were similar in WT and Cox4i2$^{-/-}$ PASMCs. Cox4i2 peptides were found only in the monomeric COX and were absent in the respirasome. Of note, hypoxia-induced reduction of ETS components (NADH, ubiquinol, cytochrome *c*) was prevented in Cox4i2$^{-/-}$ PASMCs. CMT167 cells expressing either Cox4i1, or Cox4i2 mutants lacked hypoxia-induced superoxide production, which was detected only in cells expressing WT Cox4i2. In contrast, overexpression of Cox4i1, or Cox4i2, or Cox4i2 mutants did not affect oxygen affinity.
Conclusion: Our findings suggest that Cox4i2 sensitizes the ETS to hypoxia-induced redox changes, likely by its preferential expression in the monomeric COX. The molecular role of the cysteine residues needs to be further investigated.

Abstract No. 505

Chronic Intermittent Hypoxia Leads to Disrupted Clock Gene Expression in Mouse Lung Tissues: Potential Implications for Lung Cell Senescence

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Introduction
Obstructive sleep apnoea (OSA) is a common disorder in which recurrent apnoea and hypopnoea produce repeated episodes of hypoxaemia followed by reoxygenation. We previously showed that OSA was associated with telomere shortening (*Ann Am Thorac Soc*, 2016). p21, the main effector of p53, is activated in response to DNA damage and telomere dysfunction but is down-regulated by certain clock genes, more specifically, Rev-Erbα, a central transcriptional repressor of the molecular clock.

Methods
We investigated lung-cell senescence in mice exposed to chronic intermittent hypoxia (CIH), 8 hours a day, for one to 21 days and analysed the lungs on the day after CIH discontinuation.

Results
Rhythmicity of the canonical clock genes Rev-Erbα, Rev-Erbβ, and Bmal-1 was associated with rhythmicity of lung p21 during normoxia, with opposite variations in Rev-Erbα and p21. Compared to normoxia, CIH for 1, 2, 7, or 21 days increased Rev-Erbα at ZT 2-4 and downregulated p21 in both young and old mice. No changes were observed later in the day. Senescence markers including p16 and SASP components were unaffected by CIH exposure. Bulk RNA sequencing performed 3 weeks after CIH confirmed dysregulation of the circadian clock genes (Dbp, Rev-Erbα, Hlf). Treatment of the mice with a Rev-Erbα antagonist partially restored p21 expression after 1 day of CIH, supporting a causal link between changes in lung Rev-Erbα and p21. No lung structural alterations were observed, suggesting protection afforded by p21 downregulation against cell senescence. Studies are under way to assess the consequences of CIH exposure in Rev-Erbα- and Bmal-1-deficient mice.

Conclusion
CIH has a major impact on clock gene expression, which may in turn play a protective role against cell senescence.

Abstract No. 506

IRG1/Itaconate Axis as an Immunometabolic Regulator of Pulmonary Hypertension

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Pulmonary hypertension (PH) is a progressive and lethal cardiopulmonary disorder. Bone marrow (BM)-derived hematopoietic cells, particularly myeloid cells can contribute to the process of pulmonary vascular remodeling. Immune Responsive Gene 1 (IRG1) encodes *the enzyme* that produces itaconate under inflammatory conditions in myeloid cells. However, the association of IRG1/itaconate axis on PH progression is largely unknown. Here, we reveal that following phenotypic characterization, *Irg1*$^{-/-}$ mice exhibit pulmonary vascular remodeling, cardiac hypertrophy and dysfunction in baseline. Hypoxic exposure of *Irg1*$^{-/-}$ mice, as well as adoptive transfer of BM from Irg1-deficient mice into naïve WT mice exacerbates PH phenotype and alters immune-cell composition as infiltration of macrophages and dendritic cells to the lungs. Transcriptomic profiling highlights enrichment of the pathways associated with carbon metabolism in *Irg1*$^{-/-}$ mice lungs. Due to increase in infiltrated myeloid cells population in the lung of *Irg1*$^{-/-}$ mice, we analyzed the hematopoietic stem cells (HSCs) multi-potency. Irg1-dificient mice exhibit a shift in granulocyte-macrophage progenitors (GMPs) and lymphoid primed multipotent progenitors (MPP-Ly). *In vitro* studies revealed that endogenous and exogenous activation of IRG1/itaconate axis diminish proliferation of human pulmonary vascular cells. Notably, the effect of itaconate was translated *in vivo*, where its administration attenuated PH in monocrotaline rats. Thus, our data suggest that HSCs differentiation impairment in *Irg1*-ablated mice leads to pulmonary immune-cell profile alteration. Moreover, IRG1/itaconate axis can play a regulatory role in the development of PH through an immunometabolic reprogramming of immune cells phenotype and activation.

Abstract No. 507

Mechanistic Evaluation of The Effects of Postnatal Injury on Neonatal Pulmonary Vascular Disease

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Non-physiologic shear stress (SS) and oxygen exposure contribute to bronchopulmonary dysplasia (BPD), associated with pulmonary vascular disease due to a maldeveloped vascular bed. Understanding the cellular crosstalk between the injured endothelial, smooth muscle, and circulating immune cells will help to delineate their role in BPD. To this end, we developed a millifluidic PIPE (=*Pulmonary In Vitro Perfusion*) model to mimic BPD pathophysiology and its rarefied capillary bed by applying physiologic (1 dyne/cm^2) and non-physiologic (21 dyne/cm^2) SS in the presence/absence of hyperoxia (fracture of inspired oxygen (FiO2); 0.21, 0.4, 0.8) in a triple coculture of primary human microvascular endothelial cells (hPMEC), primary human pulmonary artery smooth muscle cells (hPASMC), primary human pulmonary and monocytic (THP1) cells. Leveraging our novel model, we showed that the exposure of the hPASMC/hPMEC coculture to monocytes under non-physiologic SS enhances the cell layers' permeability, nitric oxide of ECs, total soluble collagen, and inflammatory cytokines secretion (IL8, IL6, IL1b, TNFα, TGFß, and CCL2) compared to the physiologic SS. The induction of these vascular pathology markers next to the production of reactive oxygen species is aggravated upon the exposure of the triple coculture model to hyperoxia (FiO2 0.8). In contrast, the hPASMC/hPMEC coculture alone –without monocyte circulation– did not exhibit these responses. Under non-physiologic SS, we also observed, an adhesion, extravasation, and subsequent transmigration of THP1 cells across the EC barrier significantly contribute to extracellular matrix formation. Our findings provide new insight into pathophysiologic details that could serve as therapeutic targets when aiming to reverse vascular pathology.

Contribution of a Disease-spanning Working Group: Lung Environment Interaction

Abstract No. 508

Morphomolecular analysis of the capillary network in alveolar capillary dysplasia

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Introduction: Alveolar capillary dysplasia (ACD) is a rare lung developmental disorder associated with fatal outcomes in newborns. Mutations in a transcription factor involved in the development of pulmonary capillaries, the *forkhead box F1* (*FOXF1*) gene, are causative of the disease in 60% of affected infants.

Aims and objectives: We hypothesized whether there are mechanisms beyond *FOXF1* involved in the pathogenesis of ACD. To identify these, we performed three-dimensional ultrastructural imaging combined with a comprehensive molecular work-up.

Methods: Human lung tissues from ACD, non-specific pneumonia (NSIP), and healthy controls (n=12 cases, respectively) were analyzed. Morphometrical assessment of the pulmonary vasculature was performed using transmission and serial block face electron microscopy. The interacting molecules between *FOXF1* and basement membrane proteins were analyzed using single gene sequencing and immunostaining. In addition, transcriptome profiling was performed using a self-designed panel of 435 genes and a biological pathway analysis tool.

Results: Pulmonary capillaries in ACD showed a unique pattern of altered, hypertrophied, multi-layered basement membranes surrounded by large collagen deposits. *FN1*, *COL4A1*, *CDH5*, and *VIT* were identified as genes potentially contributing to these alterations. In addition, ACD tissues showed a loss of bona fide lung capillaries and a replacement by COL15A1+ vessels as recently described in NSIP.

Conclusions: This study provides new insights into the ultrastructure and pathophysiology of ACD. Future studies are needed to explore the role of COL15A1, the effect of FN1 knockouts and the therapeutic downregulation of *FN1* overexpression in ACD.

Abstract No. 509

Multi-omics profiling unveils regulatory subgroups of PAH-associated right ventricular remodeling

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The right ventricle (RV) remodeling plays a crucial role in the functional outcome of PAH (Pulmonary Arterial Hypertension) patients. However, its underlying mechanisms in different disease states are poorly understood. The aim of this study is to investigate molecular changes underlying right ventricular dysfunction in PAH deeply.
In the first phase, we identified two subgroups in decompensated RV through an unsupervised RNAseq analysis of human samples, which were later labeled as early and late stages of maladaptation based on their similar expression pattern with MCT-induced rats. Then, by integrating with the plasma proteome of two independent cohorts, we identified a panel of five extracellular matrix proteins that could significantly classify patients with compensated vs early and late decompensated RV. In the second phase of the study, we performed multi-omic profiling to further investigate longitudinal alterations in PAH-associated RV of MCT rats. An integrative clustering and annotation using information from both modalities resulted in identifying different subtypes of fibroblasts, which significantly change during disease progression. Furthermore, through differential TF footprint analysis, we found the FOSL family of TFs as one of the vital master regulators in the conversion of fibroblasts to pro-fibrotic myofibroblasts in the early stages of maladaptive RV development in PAH.
This comprehensive multi-omics identified new subgroups of human RV hypertrophy beyond the clinical measurements, while the combination with proteome proposed a panel of potential biomarkers for PAH. Moreover, the integrative single-cell approach revealed significant upstream regulatory complexes that govern the early to late transition in PAH-associated RV remodeling.

Contribution of a Disease-spanning Working Group: Single Cell Analysis

Abstract No. 510

Characterization of Transcription factor RBPJ in Pulmonary Hypertension

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Pulmonary hypertension (PH) is a life-threatening disease, characterized by an elevated pulmonary arterial pressure due to pulmonary vascular remodeling. If left untreated, PH progresses to right heart failure and eventually death. Pulmonary vascular remodeling can be caused by chronic alveolar hypoxia triggering an uncontrolled proliferation of pulmonary arterial smooth muscle cells (PASMC) and dysfunction of pulmonary arterial endothelial cells (PAEC). Despite the already characterized involvement of Notch receptors in PH pathogenesis, involvement of downstream signaling is poorly understood. In this regard, we aim to assess the role of recombination signal-sequence-binding protein J (RBPJ; also known as CSL or CBF1) in PH pathogenesis, the central transcription factor in the Notch pathway.
The *in vivo* function of RBPJ for disease development will be assessed by using tamoxifen-inducible cell-specific gene-knockout mice in a model of chronic hypoxia-induced PH. Mechanistically, loss- and gain-of-function experiments will be performed to unravel the role of RBPJ for vascular cell dysfunction in PH.
Our data revealed a hypoxia-dependent regulation of RBPJ in primary human PASMC but not PAEC. Furthermore, hypoxia-induced PASMC proliferation was reversed to baseline conditions following *RBPJ* silencing. Preliminary *in vivo* data in cell-specific *Rbpj*-knockout mice presume a role of RBPJ in chronic hypoxia-induced PH.
In conclusion, our data provide evidence for a role of RBPJ in chronic hypoxia-induced PH in mice by affecting vascular cell dysfunction.

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Abstract No. 511

Piezo1 in Pulmonary Arterial Smooth Muscle Cells – a Critical Player for the Development of Pulmonary Hypertension

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Pulmonary hypertension (PH) is a life-threatening and progressive, but yet incurable disease. The hallmarks of PH comprise sustained contraction and excessive proliferation of pulmonary arterial smooth muscle cells (PASMCs). A major stimulus to which PASMCs are exposed during PH development is altered mechanical stress. Mechanosensitive ion channels, such as Piezo1, perceive such mechanical stimuli and translate them into various cellular responses. Thus, the objective of the present study was to elucidate the specific role of Piezo1 in PASMCs for PH-development and progression.

Taking advantage of PASMCs from idiopathic pulmonary arterial hypertension (IPAH)-patients and two mouse strains characterized by SMC-specific Piezo1 knock-out, we assessed the SMC-specific role of Piezo1 in PH-development and progression *via* experiments in isolated, perfused and ventilated mouse lungs, wire myography and proliferation assays. *In vivo* function of SMC-specific Piezo1 knockout was evaluated upon induction of chronic hypoxia-induced PH (CHPH).

Piezo1 expression and proliferation was enhanced in PASMCs from IPAH-patients and mouse PASMCs upon hypoxic exposure. SMC-specific Piezo1-deletion prevented the hypoxia-induced increase in proliferation. Moreover, Piezo1-knockout reduced hypoxic pulmonary vasoconstriction (HPV) in isolated, perfused and ventilated mouse lungs, pulmonary arteries and hemodynamic measurements *in vivo*. Consequently, Piezo1-deficient mice were significantly protected against CHPH-development with ameliorated right heart hypertrophy and improved hemodynamic function.

This study provides evidence for Piezo1 expressed in PASMCs being critically involved in the pathogenesis of PH - most likely by controlling pulmonary vascular tone and pulmonary arterial remodeling.

Abstract No. 512

Role of Uqcrh in hypoxic pulmonary vasoconstriction and pulmonary hypertension

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Background: Acute hypoxia triggers hypoxic pulmonary vasoconstriction (HPV), which is an essential mechanism that matches pulmonary arterial blood flow to alveolar ventilation to optimize systemic oxygenation. In contrast, chronic hypoxia leads to pulmonary vascular remodeling and pulmonary hypertension (PH). Mitochondria play an important role in both HPV and PH, potentially in particular reactive oxygen species originating from complex III of the respiratory chain. Therefore, we investigated the role of ubiquinol-cytochrome c reductase hinge protein (Uqcrh), an integral subunit of mitochondrial complex III, in HPV and PH development.
Methods: HPV was examined in isolated perfused and ventilated lungs from both wild-type (WT) and *Uqcrh*$^{-/-}$ mice. mRNA level of *Uqcrh* was examined in lung tissues from donors and patients with idiopathic pulmonary artery hypertension (IPAH), as well as in mouse pulmonary artery smooth muscle cells (mPASMCs) exposed to 1% O_2 for 24, 48, and 72 hours. Additionally, the protein levels of HIF-1α in mPASMCs, as well as the proliferation of these cells, were studied after hypoxic exposure.
Results: HPV was absent in *Uqcrh*$^{-/-}$ mouse lungs during acute hypoxic ventilation, while vasoconstriction induced by the thromboxane mimetic U46619 was preserved. However, there were no significant differences in the mRNA levels of *Uqcrh* in lung homogenates from donors and IPAH patients, nor in mPASMCs exposed to hypoxia for different time points. Similar to WT mPASMCs, *Uqcrh*$^{-/-}$ mPASMCs exhibited increased HIF-1α protein expression and proliferation after chronic hypoxic exposure.
Conclusions: Our preliminary findings suggest that the presence of Uqcrh is essential for HPV but not PH development.

Abstract No. 513

Osteopontin in experimental right ventricular remodeling

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Background: Right ventricular (RV) failure is a critical determinant of survival and prognosis in patients with pulmonary hypertension. Circulating levels of osteopontin (OPN) are increased in these patients and associated with RV remodeling and dysfunction. We hypothesized that OPN-knockout (OPN-KO) mice would be protected from deleterious RV remodeling, and that the reconstitution of OPN using recombinant OPN (rOPN) would abolish the effects of OPN deficiency on the RV in a mouse model of RV failure.
Methods: We performed sham or pulmonary artery banding (PAB) surgery in OPN-KO and wild type (OPN-WT) mice and administered rOPN for 3 weeks. Cardiac phenotypes were evaluated with echocardiography and catheterization.
Results: PAB surgery caused a significant increase in RV systolic pressure and RV remodeling and dysfunction in both OPN-KO and OPN-WT mice with no differences between them. Treatment with rOPN had no effect on the functional and structural parameters of the RV in either OPN-KO or OPN-WT mice. Interestingly, OPN-KO mice exhibited larger right atrium (RA) dilatation compared to OPN-WT mice, which was attenuated with rOPN treatment. Despite a similar degree of RV remodeling, PAB OPN-KO mice displayed a slightly higher mortality than OPN-WT mice, although this difference was not statistically significant. Treatment with rOPN slightly but not significantly improved the survival of these mice.
Conclusions: The lack of OPN did not affect RV remodeling in mice subjected to PAB. However, OPN deficiency was associated with a larger RA dilatation and a higher mortality in PAB mice, which were attenuated with OPN reconstitution. These results suggest that rOPN may have therapeutic potential in treating RV failure.

Abstract No. 514

Deficiency of Smooth Muscle ADAR1 RNA Editing Exacerbates Vascular Remodeling and Pulmonary Hypertension

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Adenosine deaminase acting on RNA 1 (ADAR1) catalyzes the conversion of adenosine (A) to inosine (I) in double-stranded RNA (dsRNA), which is critical to prevent auto-inflammatory responses mediated by activation of the type I interferon (IFN) signaling. Here, we define the role of ADAR1-dependent RNA editing in interferon-β (IFNβ) activation and pulmonary artery smooth muscle cell (PASMC) remodeling in pulmonary arterial hypertension (PAH), a devastating disease leading to right heart failure and premature death. PASMCs from IPAH patients displayed decreased levels of ADAR1 mRNA and protein, accompanied by reduced A-to-I editing compared to healthy PASMCs. ADAR1 knockdown in PASMCs upregulated IFNβ and IFN-Stimulated Genes (ISGs). Importantly, hypoxic *ADAR1*$^{SMC\text{-}KO}$ mice developed severe PH compared to wild type controls, as evidenced by excessive vascular remodeling in distal arterioles and increased vascular leakage resulting in elevated right ventricular systolic pressure and right ventricular hypertrophy. Mechanistically, IFNβ signaling in *ADAR1*$^{SMC\text{-}KO}$ lungs induced the recruitment of inflammatory cells, including macrophages, which in turn enhanced PASMC proliferation and muscularization. In synthesis, ADAR1-dependent A-to-I RNA editing tempers IFN signaling in PASMCs and ADAR1 deletion in PASMCs leads to increased IFNβ activity, macrophage recruitment, and exacerbated vascular remodeling, thus demonstrating a direct detrimental role of vascular innate immune responses in PH.

Abstract No. 515

Function of Th9 and IL-9$^+$ cells in pulmonary hypertension

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CD4 T cell are divided into subpopulations based on their cytokine profile, of which one newer subtype of Th cells have been implicated in tumorigenesis: Th9 cells, which predominantly produce the cytokine IL-9. Pulmonary hypertension (PH) is characterized by perivascular accumulation of T cells as well, which contributes to the vascular changes. In the PH, CD4$^+$ T cells accumulate and communicate with pulmonary arterial cells and surrounding cells via soluble cytokines and cell-derived molecules. However, it is not well known which subpopulations of T cells are involved in the pathogenic mechanism. In addition, until now, the role of Th9 cells and cytokines secreted by them have not been studied on the pathology of PH. The influence of Th9 PH has been investigated in *in-vitro* to study the functional changes in state of pulmonary arterial adventitial fibroblasts (PAAFs), pulmonary arterial smooth muscle cells (PASMCs) and pulmonary microvascular endothelial cells (PMVECs), likewise Th9 were mapped and validated on patient idiopathic pulmonary PH (IPAH) lung tissues and cells. Our data shows that Th9 and IL-9$^+$ cells are increased in perivascular compartment in lung tissues of human IPAH patients, as well as, IL-9$^+$ cells are increased in perivascular compartment upon 35 days MCT rat model and 5 weeks of hypoxic exposure of mouse lungs. In addition, IL-9 induces proliferation and migration of donor/IPAH human PASMCs and control mouse PASMCs; proliferation (but no migration) of IPAH PAAFs and angiogenesis and proliferation in human and mouse PMVECs. In summary, our study shows for the first time a role of IL9$^+$ cells in PH pathology as well a proliferative, migratory and angiogenesis effect on pulmonary vascular cells, where underlying mechanisms need to be continue studied.

Abstract No. 516

lncRNA19 - a major regulator of cellular responses to hypoxia in the lung

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Hypoxia, a condition characterized by insufficient oxygen levels, plays a crucial role in various physiological and pathological events. The transcription factor HIFα, a key player in hypoxia, dysregulates multiple signaling pathways inducing a pathological phenotype. Recent studies have shown that several long non-coding RNAs(lncRNAs) are dysregulated under hypoxia. lncRNAs can regulate several signaling pathways by acting as RNA-decoy, miRNA sponge, and splicing modulator. In this project, we aim to delineate the role of lncRNAs in modulating the hypoxia-driven pathways and their contribution to the pathogenesis of chronic lung diseases.

Screening for dysregulated lncRNAs under hypoxia was done by performing RNA-sequencing(RNAseq) from human pulmonary arterial smooth muscle cells(hPASMCs) exposed to 24hrs of hypoxia(hox). The top ten most significantly upregulated ones were further validated at different time points of hox exposure. Functional studies revealed that the knockdown of LNC19 significantly reduced the proliferation and increased the apoptosis of hox-exposed hPASMCs. Analysis of the promoter region of LNC19 revealed that it lacked the hypoxia responsive element(HRE),the binding site of HIFα. However, based on knockdown experiments, HIFα seems to regulate the expression of LNC19. Furthermore, RNAseq showed that LNC19 regulated the translation mechanism in hPASMCs. As such, the contribution of LNC19 in the IRES-dependent translation of hypoxic-responsive genes will be further investigated. Screening of LNC19 in different lung diseases revealed that it was significantly upregulated in COPD. Further studies delineating the role of LNC19 in-vitro and ex-vivo in the hypoxia-driven pathways and its therapeutic potential in COPD will be performed.

Abstract No. 517

Alteration of ferroptosis marker in pulmonary hypertension

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Introduction: Ferroptosis is a novel type of cell death regulated by the antioxidant selenoprotein glutathione peroxidase 4 (Gpx4) and is characterized by iron accumulation and increased lipid peroxidation (LPO). Inhibition of ferroptosis was found in cancer cells, therefore we hypothesize that attenuated ferroptosis may also promote pulmonary hypertension (PH).
Methods: Protein expression of Gpx4 and transferrin receptor 1 (TfR1) was determined by Western blot in lung homogenates from chronic hypoxia-exposed mice and PH patients, and in human (h) and murine (m) primary pulmonary arterial smooth muscle cells (PASMCs) after *in vitro* exposure to chronic hypoxia (1% O_2). Glutathione (GSH) levels, LPO by BODIPY 581/591 C11 staining and by lipid radical-trapping activity, and cell proliferation were evaluated in hPASMCs and mPASMCs treated with the ferroptosis inducer, erastin and exposed to 21% O_2 and 1% O_2.
Results: Protein expression of Gpx4 and TfR1 was unchanged in lung homogenates from mice exposed to chronic hypoxia compared to control mice. However, in lung homogenates from PH patients, Gpx4 protein expression was increased compared to donor lungs indicating decreased ferroptosis in PH. Erastin treatment decreased Gpx4 and increased TfR1 protein expression, inhibited cell proliferation, decreased GSH levels and increased LPO in hPASMCs and mPASMCs exposed to 21% O_2 and 1% O_2, indicating increased ferroptosis.
Conclusion: Ferroptosis markers indicated decreased ferroptosis in lung homogenate from PH patients. Ferroptosis induction by erastin treatment inhibited cell proliferation, increased LPO and decreased GSH levels in hPASMCs and mPASMCs. *In vivo* studies will be performed to investigate the role of ferroptosis inducers in the treatment of chronic hypoxia-induced PH.

Abstract No. 518

Role of MST1/2 Kinases and its therapeutic modulation in Pulmonary Arterial Hypertension

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Pulmonary arterial hypertension (PAH) is progressive disease with multiple pathologies, characterized by hyper proliferative, prosurvival and anti-apoptotic behavior of pulmonary vascular resident cells. The mechanisms underpinning the pathology of PAH is unclear.

Mammalian STE20-like protein kinases 1/2 (MST1/2) are members of HIPPO signaling axis involved in cellular proliferation and survival. Our recent report on the pro-survival/hyper proliferative role of MST1/2 signaling in PAH confirms that MST1/2 can be a potential therapeutic target for the reversal of PAH *in vivo*. Interestingly, XMU-MP-1 inhibitor is known to selectively inhibit MST1/2 kinases. Thus, this study aims to elucidate the in vitro and in vivo pharmacological modulation of MST1/2 kinases in PAH.

Our in vitro functional study shows that treatment of IPAH-PASMCs with XMU-MP-1 inhibits BrdU incorporation, migration and wound closure. Interestingly at the molecular level, significant downregulation of Hippo signaling molecules is associated with re-activation of BMPR2 and SIRT1 are observed upon XMU-MP-1 treatment as well as with MST1/2 knockdown.

Furthermore, daily intraperitoneal administration of XMU-MP-1 to monocrotaline – induced PAH rats attenuated mean pulmonary arterial pressure and improved cardiac output. Also, XMU-MP-1 dysregulated hippo signaling molecules and significantly ameliorated pulmonary vascular remodeling and collagen deposition in pulmonary arteries as well as collagen deposition in the RV of XMU-MP-1 treated MCT-PAH rats.

From our data gathered so far, pharmacological modulation of MST1/2 kinases could be a potential therapeutic strategy in PAH.

Abstract No. 519

In vitro modeling of heritable pulmonary arterial hypertension with induced pluripotent stem cell-derived endothelial cells and smooth muscle cells

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Heritable pulmonary arterial hypertension (HPAH) is a progressive disease with irreversible pre-capillary pulmonary artery remodeling and increased arterial pressure associated with severe cardiovascular and respiratory symptoms. Mutations in the bone morphogenetic protein receptor 2 (BMPR2) gene predispose for dysfunctional arterial endothelial cells (ECs) and smooth muscle cells (SMCs), but knowledge about the molecular mechanisms and additional triggers assumed in HPAH pathogenesis is limited. Patient-specific induced pluripotent stem cells (iPSCs) offer an attractive cell source for generating functional iECs and iSMCs or even vascular organoids for *in vitro* disease modeling of HPAH.

Two HPAH-iPSC lines were successfully generated from HPAH patients with different mutations in the BMPR2 gene and used for *in vitro* modeling of HPAH. HPAH-iSMCs showed decreased contractile properties in a collagen contraction assay and decreased contractile markers compared to WT-iSMCs. In long-term cultures, pro-proliferative abilities were observed in HPAH-iSMCs, indicated by high KI67 levels, suggesting an imbalance between the contractile and synthetic phenotypes. HPAH-iEC lines proliferated more intensely and appear to lose their EC properties. A secondary trigger of HPAH seems to be critical oxygen deprivation (hypoxia), which caused an increase in mesenchymal markers in HPAH-iECs in a hypoxia chamber experiment. This possibly indicates an endothelial-to-mesenchymal transition. Cell aggregates containing both HPAH-iECs and iSMCs served as the basis for a vascular organoid model that provided initial evidence of an altered angiogenesis in a sprouting assay. Refinement of the organoid model may reveal the impact of BMPR2 mutations on HPAH pathology and novel therapeutic targets.

Abstract No. 520

Mesenchymal stem cells as a therapeutic option for BMPR2-mutation induced PAH: an *in vitro* study using hiPSCs as tool for disease modelling

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Heterozygous germline mutation in BMPR2 gene is the major causative factor for FPAH. BMPR2-mutation induced PAH is managed symptomatically in the clinic but there is no curative therapy, until now. We created heterozygous BMPR2-mutation in hiPSC cell line using CRISPr-Cas9 method. We generated endothelial cells (iECs), smooth muscle cells (iSMCs) and also 3D vascular organoids from both wild type (parental) and from BMPR2-mutated hiPSC line, as well as, from patient-derived hiPSCs, and studied them under different experimental conditions. MSCs were isolated from human umbilical cords (hUC-MSC) and were also generated from WT and BMPR2-mutated hiPSCs. Endothelial cells and smooth muscle cells generated from BMPR2-mutated hiPSCs showed increased cell proliferation, resistance to apoptosis in the presence of TGF-beta, altered cell adhesion to ECM and increased expression of pro-inflammatory cytokines. EndMT markers were also significantly upregulated in BMPR2 mutated iECs when compared to the WT iECs. BMPR2-mutated vascular organoids showed defective vasculogenesis which can be restored using mesenchymal stem cell co-culture. Cross-co-culture of iMSCs derived from WT and BMPR2-mutated hiPSCs with WT and BMPR2 mutated vascular spheroids improved and diminished the vasculogenic response, respectively. Conditioned media from MSCs also showed immunomodulatory properties in human macrophages. hiPSCs are excellent *in vitro* tool for BMPR2-mutation induced PAH disease modelling during early development, and our model system using adult stem cells and hiPSCs co-culture might help in understanding the therapeutic effect of MSCs in BMPR2-mutation induced PAH.

Abstract No. 521

The lung microvasculature promotes Alveolar Type 2 cell differentiation via secreted SPARCL1

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The mammalian lung is highly vascularized to allow efficient oxygenation of the blood. Despite the prevailing image of lung capillaries as passive conduits for blood, they associate with the airway epithelium before birth, suggesting an instructive role on neighboring cells. Focusing on factors secreted by the microvascular endothelium in late embryonic stages, we screened 6 recombinant candidate proteins using fetal alveolar organoids. We identified SPARCL1 as an extracellular matrix-associated molecule capable of promoting AT2 cell differentiation *in vitro*. SPARCL1 induced a dose-dependent increase in *Lyz2* transcription in organoids and it was downregulated in hypovascular lungs. Genetic evidence from *Fgf10*/*Sparcl1* compound mutants indicates that Sparcl1 does not synergize with FGF signaling for AT2 cell differentiation. We hypothesize that SPARCL1 alters the cell cycle progression of alveolar epithelial progenitors, similar to its reported function in endothelial cells. Our data suggest a novel function of SPARCL1 in respiratory cell differentiation that could be exploited to promote lung maturation in premature infants.

Abstract No. 522

Isolation and characterization of human pulmonary microvascular endothelial cells - Development of a human cell biobank

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Introduction: Differentiation and dysfunction of the pulmonary microvasculature play an important role in the pathogenesis of various pulmonary diseases such as pulmonary fibrosis, end stage pulmonary hypertension, and pulmonary veno-occlusive disease. Most studies so far have either used animal models or commercially available human pulmonary microvascular endothelial cells. Human endothelial cell isolation and cultivation pose an important tool to gain understanding of the development and treatment of severe pulmonary diseases.
Methods: Tissue samples removed from either lung explants or surgical resections were obtained from 13 patients of varying severe lung diseases as well as from 2 healthy / non-malignant controls. The pleura, large blood vessels and airways were removed before mechanical dissociation and enzymatic digestion. After straining, the cell suspension was plated on collagen-coated 6 well plates. Human endothelial cells were selected utilizing two CD31 magnetic beads separation steps. Human endothelial cells were characterized by CD31- and CD90-flow cytometry after 21 days of cultivation and cryo-preserved from passages 5-7.
Results: Here we demonstrate a successful method to isolate and characterize primary cultures of pulmonary microvascular endothelial cells from different end-stage pulmonary diseases including healthy and non-malignant tissues. Human pulmonary microvascular endothelial cells exhibited characteristic cobblestone morphology with high expression of endothelial cell markers (CD31, VE-Cadherin and vWF) and non or low expression of mesenchymal markers (CD90). With this method up to 20 x 10^6 cells per patient were cultivated and cryo-preserved. These cells were successfully revitalized and used for further experiments.

Abstract No. 523

Combined assessment of lung morphology and function using photon-counting CT (PCCT): initial experience in patients with chronic thromboembolic pulmonary hypertension (CTEPH)

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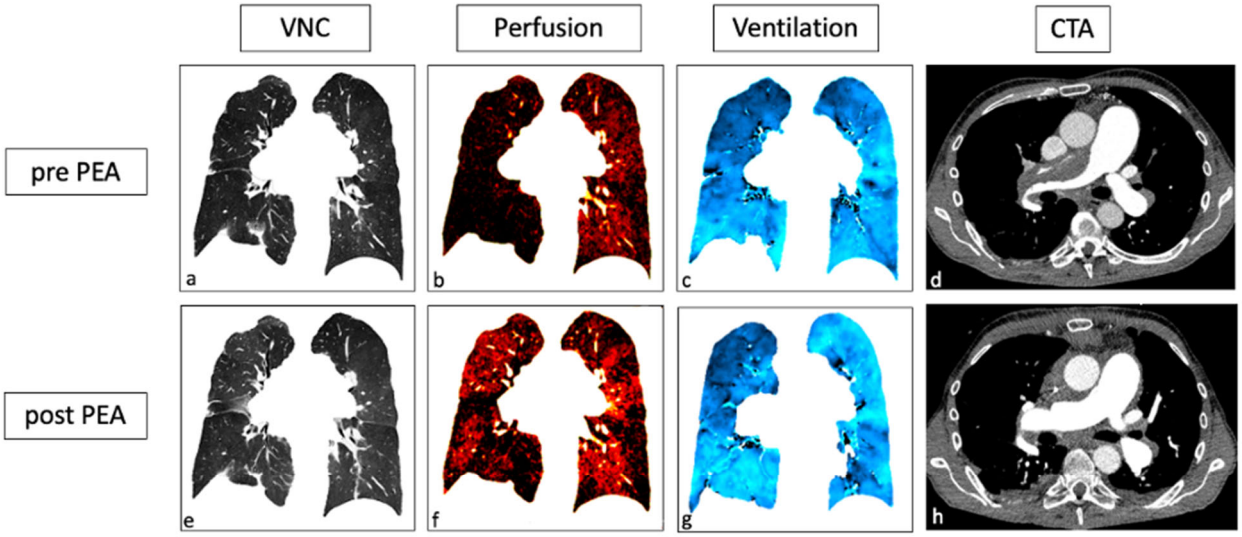
Objectives
Assessment of regional ventilation and perfusion as well as lung structure with PCCT in patients with CTEPH.

Methods
Five patients with CTEPH underwent a ventilation/perfusion CT scan before and after Pulmonary endarterectomy (PEA).
Contrast-enhanced CT scans in inspiration and expiration were performed. Iodine maps were generated as a surrogate for perfusion. Non-linear registration of the inspiration/expiration scans was performed, and a regional ventilation image was calculated. Statistical analysis included the calculation and comparison of lobe-wise mean values of ventilation and perfusion at both pre- and post-operative time points.

Results
The main thrombus burden was predominantly in the right lung. Preoperatively, the iodine maps showed focal and lobar perfusion defects, while there were no relevant ventilation deficits in the corresponding areas (mismatch). Mean perfusion increased significantly in the treated lobes of the right lung, whereas mean perfusion remained largely stable in the left lung (right upper lobe: + 16.82%, middle lobe: +67.18%, right lower lobe: +77.96%, left upper lobe: -9.03%, left lower lobe: -7.57%).

Conclusion
PCCT may provide a comprehensive, single-modality assessment of patients with CTEPH



Perfusion and ventilation images and CT angiography of a patient with **chronic thromboembolic pulmonary hypertension (CTEPH)** before (upper row) and after (bottom row) pulmonary endarterectomy (PEA).
There was no emphysema in the VNC images. Before surgery, there was a central thrombus in the right pulmonary artery with a corresponding multi-lobar perfusion defect (d,b). Preoperatively, there was a V/Q mismatch in the right lung. Postoperatively, the perfusion increased in the right lung (f). Ventilation remained stable in pre-a and postoperative PCCT scans (c,g).

Abstract No. 524

Role of carbonic anhydrases 9 and 12 in pulmonary hypertension

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Carbonic anhydrases (CAs) are family of enzymes that reversibly catalyze carbon dioxide hydration to bicarbonate and protons. Elevated expression of CA9 and 12 isoforms have been reported in various types of cancer. Pulmonary vascular remodeling, a hallmark of pulmonary hypertension (PH) has been characterized by several cancer-like features, such as dysregulated proliferation and migration. Therefore, we hypothesize that CA9 and 12 play an important role in the pathogenesis of PH.
Our initial investigation showed increase of CA9 and 12 mRNA and protein expression in the lungs of patients with idiopathic pulmonary arterial hypertension (IPAH), compared to donors. Immunohistochemical analysis demonstrated that CA9 and 12 are indeed expressed in pulmonary vasculature. Furthermore, there was a significant increase in CA9 and 12 expression in human pulmonary artery smooth muscle cells (PASMCs) after hypoxia and non-hypoxia stimulation. Additionally, using the small molecule inhibitor we have shown the involvement of CA9 and 12 in the proliferation and migration of human PASMCs. Maintaining optimal intracellular pH is a crucial event following the metabolic shift from oxidative phosphorylation to glycolysis, feature shared in both cancer and PH. By pharmacological inhibition, we have demonstrated important role of CA9 and 12 in human PASMCs intracellular pH homeostasis. In addition, CA9 and 12 inhibition lead to the significant decrease in acidification of PAMSCs extracellular milieu.
Therefore, our data indicates CA9 and 12 elevated expression and important role in function of pulmonary vascular cells in pulmonary hypertension, highlighting carbonic anhydrases as potential players in the pathology of this severe and life-threatening pulmonary vascular disease.

Abstract No. 525

Obesity impacts hypoxia adaptation of the lung

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Obesity is mostly associated with adverse health consequences, but may also elicit favorable effects under chronic conditions. This “obesity paradox” is under debate for pulmonary diseases. Since confounding factors complicate conclusions from human studies, this study utilized a controlled animal model combining diet-induced obesity and chronic hypoxia as model for pulmonary hypertension and COPD.
Male C57BL/6 mice were fed control or high fat diet for 30 weeks, half of the animals were exposed to chronic hypoxia (13% O_2) for 3 weeks.
Hypoxia induced right ventricular hypertrophy, thickening of pulmonary arterial and capillary walls, higher lung volumes, and higher hemoglobin levels irrespective of the body weight. In contrast, lung proteomes differed substantially between lean and obese hypoxic mice. Many of the observed changes were linked to vascular and extracellular matrix (ECM) proteins. In lean hypoxic animals, circulating platelets were reduced and abundances of various clotting-related proteins were altered, indicating a hypercoagulable phenotype. Moreover, airspaces were significantly distended pointing to lung hyperinflation. These differences were mostly absent in the obese hypoxic group. However, the obesity-hypoxia combination induced the lowest blood CO_2 concentrations, indicating hyperventilation for sufficient oxygen supply. Moreover, endothelial surface areas were increased in obese hypoxic mice.
Thus, obesity exerts differential effects on lung adaptation to hypoxia, which paradoxically include adverse, but also rather protective changes. These differences have a molecular basis in the lung proteome, and may influence pathogenesis of lung diseases. This should be taken into account for future individualized prevention and therapy.

Abstract No. 526

Genetic findings in a family with familial spherocytosis and pulmonary hypertension

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Pulmonary arterial hypertension (PAH) and hereditary spherocytosis can be caused by different genetic mutations. While PAH is rare, hereditary spherocytosis is a common inherited hemolytic disease with a substantial number of asymptomatic cases. In hereditary spherocytosis mutations alter the red blood cell membrane resulting in spherical, dysfunctional red blood cells leading to anemia, splenomegaly, skin jaundice and cholelithiasis. In a family with 3 cases of PH and additional spherocytosis we aimed to investigate the underlying disease. Whole exome sequencing of 4 family members was performed including the index patient who manifests both PH and hemolytic anemia due to spherocytosis, his daughter with hemolytic anemia only, his healthy son and healthy wife. No pathogenic variant in any genes known for PAH could be detected. Instead, a missense variant c.2204C>T p. (Ala735Val) was identified in the gene *Solute Carrier Family 4 Member 1* (*SLC4A1*) causative for hereditary spherocytosis. The variant was identified in the index patient, his affected daughter and his clinically healthy son. It was absent in the healthy wife of the index patient. The variant was characterized inconclusive according to ACMG guidelines. Sanger sequencing of *SLC4A1* revealed the variant segregated in two further affected family members but also in two healthy relatives. The identified variant in *SLC4A1* might be causative for the spherocytosis in this family. All PH patients had undergone splenectomy prior to the onset of the disease. Therefore, PH in this family has been classified as group V, due to hematological disorders.

Abstract No. 527

Identification of the origin of pulmonary hypertension-associated ACTA2$^+$ vascular smooth muscle cells

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JLU

Pulmonary vascular remodeling underlying pulmonary hypertension (PH) leads to the appearance of alpha-smooth muscle actin (ACTA2$^+$) cells in normally non-muscularized distal pulmonary arterioles and expansion of the tunica media of the proximal arterioles. Although the origin of these newly formed vascular smooth muscle cells (VSMCs) has gained increasing interest in recent years as it contributes to the overall understanding of PH pathogenesis, the precise origin of these PH-associated VSMCs appearing in non-muscularized vessels in the context of pulmonary hypertension induced by chronic hypoxia (HOX) is still unclear.
Therefore, the aim of the present project is to investigate which population of ACTA2$^+$ cells is the origin of the VSMCs proliferating during PH. In this experiment, 8-24 weeks old males and females *ACTA2*$^{Cre\text{-}ERT2}$*; tdTomato*^{flox} mice were exposed to normoxia or hypoxia for 28 days. Hemodynamic measurements and right ventricular hypertrophy assessment conformed PH development upon HOX exposure. We have identified nine different subclusters of ACTA2$^+$ cells, two of which distinctly respond to hypoxia conditions. Combining the FACS and scRNA-seq data, we found that the pre-existing ACTA2$^+$ cells are not the only source of newly formed ACTA2$^+$ cells, and other cellular origins of those cells are highly probable.
In future studies, we will focus on the same analysis of the ACTA2$^+$ cell population captured during the hypoxia injury. The turning point of this research will be the use of the Dre-ERT2 mice model to better characterize the ACTA2$^+$ cell population.

Contribution of a Disease-spanning Working Group: Single Cell Analysis

Abstract No. 528

The role of Tbx4 in lung disease and development

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The incidence of paediatric pulmonary arterial hypertension (PPAH), a fatal disease, is rising. PPAH and pulmonary arterial hypertension (PAH) in adults share characteristics. According to studies, a mutation in bone morphogenetic protein receptor 2 (BMPR2) may result in the development of PAH in adults. PPAH is thought to be primarily caused by a mutation in the TBX4 gene. Mutations in BMPR2 that increase the proliferation of smooth muscle in pulmonary vessels, resulting in pulmonary microvascular remodeling, are regarded as the dominant cause of PAH in adult patients. Nonetheless, it remains unclear how the PPAH is evolving. To investigate PPAH in-depth, mice with a common mutation observed in PPAH patients were created. Mice with Tbx4 mutations exhibit heart hypertrophy and limb deformation similar to PAH patients, indicating the development of PAH. However, we did not observe vascular remodeling in the pulmonary vessel, and additional 3D volumetric analysis revealed that the number of vessels in Tbx4 mutant mice was decreasing. Interestingly, there are no indications that alveolarization has been compromised. We subsequently examined the expression of Tbx4 in diverse cell types using single-cell sequencing. Tbx4 is highly expressed in mesenchymal cells, of which pericytes are responsible for the formation and stability of blood vessels. We demonstrated by means of FACS, 3D high-resolution microscopy, and single cell sequencing that the number of pericytes in Tbx4 mutant mice is significantly decreasing. A decreased number of pericytes in Tbx4 mutant mice may inhibit the formation of new blood vessels during lung development, and a reduction in the number of blood vessels may contribute to the onset of PAH.

Contribution of a Disease-spanning Working Group: Single Cell Analysis

Abstract No. 529

Role of TGFβ regulated long non coding RNA, VIM-AS1, in Pulmonary Hypertension

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Dysregulation of various long noncoding RNAs (lncRNAs) are emerging as major drivers of disease induction and pathology. An imbalance in the TGFβ and BMP signaling plays a crucial role in PH pathogenesis. The main aim of this study is to delineate the role of TGFβ regulated lncRNA vimentin antisense 1 (VIM-AS1) in PH. The expression of VIM-AS1 was observed to be significantly upregulated in the IPAH PASMCs and further induced upon stimulation with TGFβ the expression of VIM-AS1 in a concentration dependent manner. Silencing VIM-AS1 using GapmeRs significantly reversed the pro-proliferation and anti-apoptotic phenotype of IPAH PASMCs. VIM-AS1 was majorly localized in the nuclear compartment compared to the cytoplasm in IPAH PASMCs. From the RNA-seq dataset, it was observed that silencing VIM-AS1 severely affected the TGFβ-BMPR signaling pathway along with several other pathways. From the ATAC-seq dataset, the chromatin accessibility was severely altered upon VIM-AS1 knockdown in IPAH PASMCs, suggesting that VIM-AS1 can act as an epigenetic modulator. Previous studies have shown that VIM-AS1 acts an epigenetic modulator by forming R-loop at the promoter region of vimentin and altering the methylation status. Interestingly, similar to the previous study VIM-AS1 was observed to modulate the DNA methylation status of the vimentin promoter region leading to induction of gene expression. In PH, epigenetic alterations such as DNA methylation is known to play a major role in disease progression. DRIP-seq will be performed to investigate the VIM-AS1 mediated R-loop formation and its role in modulating the chromatin accessibility of TGFβ signaling genes under the disease condition. Further, the role of VIM-AS1 will be investigated in the effects mediated by sotatercept treatment.

Abstract No. 530

Investigation of interleukin-6 and interleukin-13 as novel potential therapeutic targets for the treatment of pulmonary vasculopathy in experimental systemic sclerosis

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Background:
Systemic sclerosis is a chronic autoimmune disease characterized by inflammation, fibrosis and vasculopathy. Previously, we demonstrated that functional autoantibodies against angiotensin II receptor type 1 (AT1R) play an essential role in the pathogenesis of the disease by mediating the development of skin inflammation and fibrosis as well as inflammation in the lung. Furthermore, autoimmunity to AT1R in conjunction with overexpression of the TH2 cytokine IL-13 mediates pulmonary vasculopathy in mice. Interestingly, the level of IL-6 in BAL fluid was significantly elevated and positively correlated with the severity of inflammation and vasculopathy. Therefore, we hypothesized that IL-13 and IL-6 are central players and potential therapeutic targets for pulmonary vasculopathy in this novel mouse model.

Method:
IL-13 transgenic (IL-13tg) mice overexpressing IL-13 under control of the human CD2 promoter were immunized with human AT1R and treated with neutralizing monoclonal antibodies against IL-6, IL-13 or isotype control respectively. Seven weeks after the first immunization, mice were sacrificed and tissues were analyzed for pathological changes.

Results:
Compared with the isotype control, neutralizing antibody against IL-13 inhibited the development of pulmonary vasculopathy in immunized IL-13tg mice. Unexpectedly, a neutralizing monoclonal antibody against IL-6 significantly promoted the development of occlusive vasculopathy in the lung of both immunized and non-immunized IL-13tg mice.

Conclusion:
The findings suggest a causal role of IL-13 and a protective role of IL-6 in the development of pulmonary vasculopathy in this mouse model.

Abstract No. 531

Caspase-6 is necessary and specific for morphological adaptation specifically to shear stresses in pulmonary artery endothelial cells *in vitro*

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Caspases are proteases thought to be regulators and effectors of cell death and inflammation. New research reveals they may have further unidentified functions. Our goal was to identify potential roles of caspases in morphological adaptability of endothelial cells subjected to known morphology-modulating stresses *in vitro*.

First, we subjected human pulmonary artery endothelial cells (PAEC, n=3 pooled) to a constant, unidirectional shear stress of 15 dyn/cm^2 in an *in vitro* fluid flow system for 72h. All known caspases were examined for modulation of endothelial morphological elongation parallel to flow through the addition of irreversible selective caspase inhibitors. We found morphological adaptation was functional in all situations, except with caspase-6 inhibition (74.8% cells adapted vs. 20.5% cells adapted, p=0.02).

To determine if caspase-6 necessity for shear-induced elongation was shear-specific, generally mechano-specific, or non-specific, we utilized a number of elongation-driving stimuli under caspase-6 inhibition: *in vitro* cyclic stretch at 5% and 1 Hz for 24h; micropatterned slides with thin RGD motif lines; 10 ng/mL TNF-α for 24h. In all cases, caspase-6 inhibition did not impair morphological adaptation.

Finally, caspase-6 inhibition had no effect on cell adhesion and directed migration after the induction of an electrical or mechanical wound, indicating no role of caspase-6 in functional adhesive and migratory capabilities.

Our findings suggest caspase-6 plays a vital role in allowing PAEC to adapt to fluid shear stress specifically. This finding may have important implications for developing new targeted therapeutic approaches for cardiovascular diseases characterized by endothelial damage due to increased mechanical shear stress (e.g. reperfusion injury).

DA Regeneration and Organ Replacement: Abstract No. 601 – 620

Abstract No. 601

Ferroptosis-driven ischemia reperfusion injury in lung transplantation

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Lung transplantation (LTx) is the only treatment option for the increasing number of end stage lung diseases such as COPD. Unfortunately, LTx has a 5-year-survival rate of only 56% and is significantly lower compared to other solid organ transplantations. Besides various challenges, in 20% of LTx ischemia reperfusion injury (IRI) in part causes life-threatening transplantation failure and is a major reason for primary graft dysfunction (PGD), associated with mortality. Recent studies have shown that ferroptosis, an iron-dependent regulated cell death mechanism, is a common underlying mechanism of several lung diseases, including lung IRI, making it imperative to investigate its regulatory mechanism and understand its role in IRI before, during and in the initial phase after LTx. With our research we aim to discover ferroptotic biomarkers to be able to identify at-risk individuals and develop anti-ferroptotic strategies in order to prevent PGD and graft rejection in LTx.

To gain an extensive understanding of the molecular mechanisms in pulmonary IRI, we have developed a murine orthotopic left LTx model including donor lung preparation with 30min of cold ischemia, followed by a reperfusion time course after syngeneic transplantation into the recipient. (Oxi-) lipidomics will be detected based on high resolution mass spectrometry and compared with patient-derived LTx tissue sections to identify unique ferroptotic signatures. The influence of cold ischemia will be studied and compared to warm ischemia to investigate underlying molecular mechanisms. Furthermore, a reversible left lung occlusion will also be conducted as an additional murine lung IRI model, to ultimately offer new anti-ferroptotic strategies for clinical translation and to protect patients from LTx rejection.

Abstract No. 602

Probe-based confocal laser-endomicroscopy in lung transplant recipients

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Introduction: Chronic lung allograft dysfunction (CLAD) is the major cause of morbidity and mortality after lung transplantation. Detection of subclinical abnormalities in lung allografts may allow earlier and successful interventions. Probe-based laser endomicroscopy (pCLE) allows visualisation of lung abnormalities at the alveolar and even cellular level and thus potentially detects pre-clinical manifestations of CLAD.
Method: We applied pCLE systematically in 20 lung transplant recipients who undervent surveillance bronchoscopy. Two segments of the lower and the upper lobes were analysed with pCLE and the videos obtained were scored by a blinded reader. Five different domains were scored (0-3): Distortion; Density; Cellularity; Consolidation; Bronchioli. For each domain the arithmetic mean and standard deviation was calculated. The study was approved by the LMU ethics committee.
Results: 20 Patients were included in this pilot study (3 female, age 61±4 yrs). Although none of the patients showed acute rejection on histology of transbronchial biopsies, there were abnormalities detected in pCLE (see table).

Distortion	Density	Cellularity	Consolidation	Bronchioli
1.22 ± 0.59	1.03 ± 0.58	1.05 ± 0.84	1.32 ± 0.51	1.57 ± 0.46

pCLE Scores (arithmetic mean ± SD)

Interestingly we found a correlation between the extent of architectural distortion and decline of FEV1 during follow-up (r=0.28; p=0.025).
Conclusion: pCLE offers a new approach of detecting subclinical lung abnormalities which may be related to deterioration of lung function in lung transplant recipients. Further studies are necessary to confirm these preliminary results.

Abstract No. 603

Distribution of Myofibroblast in Patients with End-Stage Lung Disease

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Myofibroblasts are the main effector cells in fibrotic remodeling and thus play a major role in the pathogenesis of end-stage lung disease (ELD). There is neither a generally accepted classification of myofibroblastic lesions, nor a histomorphological definition of key concepts like fibroblastic foci (FF). Therefore, we analyzed the distribution of FF and interstitial myofibroblastic aggregates (IMA) in human lung explants in order to compare quantitative and qualitative similarities and differences among different groups of ELD and to establish their basic definition criteria. We re-assessed the surgical specimens of 630 patients from the Institute of Pathology at Hannover Medical School with the diagnosis of ELD. In the histopathological analysis, the perpendicular orientation of myofibroblasts in the accumulation was considered obligatory for the definition of FF. Further criteria were divided into major (MaC) and minor (MiC) denominators. MaC included the alignment of myofibroblasts in a fascicular order, the immature extracellular matrix in which myofibroblasts are embedded, and the protrusion of the lesion into the alveolar space. MiC included the covering of the lesion by abrerrant hyperplastic type-II-pneumocytes or bronchial epithelium, localization of the lesion at the forefront of fibrotic remodeling, directly adjacent to ventilated airspaces, and swelling of the lesion's extracellular matrix. Lesions were designated as incomplete FF if at least 1 MaC and 1 MiC were missing. Criteria for IMA included interstitial accumulation of myofibroblast without contact with the alveolar space or airways without protrusion of the lesion. Despite the highest FF-count in UIP-IPF (up to 29 FF\cm^2) in our results, the FF were not unique to this entity.

Abstract No. 604

Machine-learning based BAL cytology of lung transplant patients

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Introduction: Long-term survival after lung transplantation (LTX) is severely limited by the poorly understood chronic lung allograft dysfunction (CLAD). Previous studies identified bronchoalveolar lavage (BAL) neutrophilia and eosinophilia as risk factors for CLAD development. Usually, BAL differential cytology is manually assessed, which is time-consuming and examiner dependent. We utilized a machine-learning based BAL cytology computer vision (BAL-ML) model to generate differential cytology of BAL cytospins of LTX patients.
Methods: 575 Diff-Quik-stained BAL cytospins of LTX patients were imaged using a Zeiss AxioScan slide scanner. Manual cell segmentation and differential cell labeling for the ground truth generation was performed by two respiratory physicians independently. The labeled training data contained representative as well as granulocyte- and lymphocyte-enriched image-subsets.
Results: Preliminary Mask-RCNN based models achieved a performance comparable to human interrater reliability in macrophage, lymphocyte and granulocyte evaluation. Challenges in imbalances in cell distributions were seen using representative training image data only. These were overcome by the addition of granulocyte- and lymphocyte-enriched training dataset.
Conclusions: BAL differential cytology by BAL-ML models is feasible and accurate. Naturally occurring label imbalances in cytospin image data can be overcome by oversampling granulocyte and lymphocyte occurrences in the training data. BAL-ML models will provide deeper insights into associations between differential cytology and morphometric features of BAL cytology with CLAD development in lung transplant patients. In future, validated models could assist BAL cytology in clinical routine.

Abstract No. 605

Flotillins are required for the cholinergic control of ATP-mediated IL-1β release

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Objective: The pro-inflammatory cytokine interleukin (IL)-1β contributes to the pathogenesis of diverse pulmonary disorders. We identified a cholinergic mechanism that inhibits the ATP-mediated IL-1β release by human mononuclear phagocytes via metabotropic signaling of nicotinic acetylcholine receptors (nAChRs), activation of endothelial NO synthase and inhibition of the ATP-sensitive P2X7 receptor. Here, we investigate if the lipid raft-associated flotillins are involved in this pathway.
Methods: Human monocytic THP-1 cells, THP-1 cell-derived macrophages, murine peripheral blood mononuclear cells (PBMCs) and bone marrow-derived macrophages (BMDMs) isolated from wild-type and flotillin-1 (*Flot1*) and/or -2 (*Flot2*) gene-deficient mice were used. The ATP-induced release of IL-1β was studied in the presence and absence of nAChR agonists. The involvement of nAChRs was tested using the specific antagonistic conopeptides ArIB (for subunit α7) and RgIA4 (for subunits α9/α10).
Results: Agonists of nAChRs efficiently inhibit the ATP-mediated IL-1β release by monocytic cells and macrophages and depends on nAChR subunits α7, α9/α10. When investigating the ATP-induced IL-1β release by murine PBMCs and BMDMs, nAChR agonists efficiently inhibited the ATP-induced IL-1β release by cells from wild-type mice. This inhibitory effect was completely reversed in in mice with a double-deficiency in *Flot1* and *Flot2* and partially reversed in cells from *Flot1* or *Flot2* gene-deficient mice.
Conclusion: Flot1 and 2 are required for the cholinergic control of ATP-mediated IL-1β release by mononuclear phagocytes. We suggest that flotillins, nAChRs, endothelial NO synthase, and the P2X7 receptor interact in lipid rafts to control IL-1β release. The exact molecular mechanisms deserve further investigation.

Contribution of a Disease-spanning Working Group: Lung Environment Interaction

Abstract No. 606

Assessment of clinical and lung function parameters of normo- and hypercapnic patients with end-stage lung disease requiring non-invasive ventilation

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Background: Elevated CO_2 levels (hypercapnia) are frequently observed in patients with chronic lung diseases. Non-invasive ventilation (NIV) with bilevel positive airway pressure (BiPAP) is often used to treat this condition, particularly in the setting of end-stage lung disease. Here, we examined clinical data and lung function parameters in patients who developed normocapnia or remained hypercapnic upon home BiPAP therapy.
Methods and results: We retrospectively analysed clinical data from 126 patients who received BiPAP therapy at the University of Giessen Lung Center between 2018 and 2022. Data were collected during routine clinic visits. Patients were classified into normocapnia (NC) and hypercapnia (HC) groups based on partial pressure of carbon dioxide (pCO_2) in capillary blood samples and CO_2 levels determined by capnography, using a cut-off value of 45 and 50 mmHg, respectively. Despite consequent use of BiPAP therapy, 42 patients remained at least mildly hypercapnic. Our results show that whereas forced expiratory volume (FEV1) and airway resistance (sRtot, sReff) markedly improved in patients who reached normocapnia upon BiPAP, airway resistance and residual volume (RV) were significantly higher in patients who remained hypercapnic and strongly correlated with pCO_2 levels. Moreover, we found that the presence of pulmonary fibrosis, long-term oxygen therapy, and diabetes significantly increased the relative risk of developing hypercapnia under BiPAP therapy.
Conclusions: Our data suggest that despite consequent NIV therapy approximately one third of patients with end-stage lung disease remain hypercapnic. As already mildly elevated pCO_2 levels are clearly associated with worse lung function, reaching normocapnia upon BiPAP therapy may be of utmost importance.

Abstract No. 607

Identification of pathway-level modules in L1000 data for drug repurposing.

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The L1000 dataset is a large-scale gene expression profiling database that measures the transcriptional response of human cells to various perturbations. The L1000 dataset provides a powerful tool for identifying potential drug targets, predicting drug efficacy, and discovering new therapeutic uses for existing drugs. However, the use of L1000 datasets for drug repurposing also has some limitations owing to the data's methodology and sparsity. Idiopathic Pulmonary Fibrosis is an end-stage lung disorder that progressively worsens quality of life and is often associated with high mortality. By leveraging L1000 dataset to query for pathway-level alterations and cross comparing with pathways in patients we identify key pathways that can be targeted to alter the disease state. Our preliminary results indicate that pathways associated with cholesterol metabolism, TGF-beta regulation of extracellular matrix, and IRAK4 pathway are interesting targets for IPF that need further testing. Finally, we propose a strategy for leveraging pathway level changes as a better metric for repurposing than gene level alterations in the case of L1000 data.

Abstract No. 608

Data-driven Transcriptome Analysis Provides Insights into the Unique Expression Profiles of Macrophages from Healthy Young Adults, Highlighting the Overexpression of Metallothionein (MT) Genes

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Pleural macrophages play a crucial role in initiating and resolving inflammation, as well as in tissue repair and regeneration. Consequently, a detailed study of pleural macrophages enhances our understanding of immune processes and cellular mechanisms within the pleural cavity.
However, most current studies of pleural macrophages rely on either cultivated cells induced by specific growth factors or cytokines from precursors, or physiological cells isolated from pathological procedures such as pleural exudates in lung cancer patients. In-depth exploration of human-isolated pleural macrophages under non-inflammatory conditions has been challenging, if not impossible, due to the scarcity of biological material.
We have employed a procedure to isolate and characterize pleural monocytes/macrophages (plMo/Mϕ) from healthy adolescents undergoing chest deformity correction. RNA-sequencing (RNA-seq) was applied to compare plMo/Mϕ with human monocyte-derived macrophages (MDMs) in their natural state (MDM-0) or IL-4-polarized (MDM-IL4). We observed that the expression of genes belonging to the metallothionein (MT) family is significantly higher in plMo/Mϕ than in other conditions. Integration with public single-cell RNA datasets from healthy bronchoalveolar lavage (BAL) data reveals that this pattern is associated with a specific cluster of macrophages, which might be responsible for controlling physiological (zinc, selenium, copper) and harmful (cadmium, mercury, arsenic) ions. Our results provide additional insights into high MTs-expressing macrophage subsets in the pleural fluid of healthy young adults and offer a detailed comparison of transcriptomic profiling of these cells over MDMs generated by traditional protocols.

Abstract No. 609

The pleural microbiome in lung transplant recipients and non-transplant patients

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Introduction:
Pleural effusions of unknown etiology frequently occur in lung transplant (LTx) recipients and are associated with reduced survival. The pulmonary microbiome is increasingly recognized in LTx recipients. The pleural microbiome however, has not yet been investigated and several factors, e. g. open chest surgery, severe immunosuppression and long-term macrolide therapy might alter the pleural microbiome in LTx recipients.

Methods:
We analyzed bacterial community structure using a cohort of 52 pleural effusions of lung transplant recipients and non-transplant patients as well as 14 matched bronchoalveolar lavage samples of lung transplant recipients by a 16S rRNA gene based metabarcoding approach.

Results:
Significant differences in pleural cytology as well as alpha- and beta-diversity were found between early and late pleural effusions after LTX. Late pleural effusions after LTX showed a trend towards a higher alpha- and beta-diversity of bacteria compared to non-transplant effusions. The pulmonary microbiome of BAL samples showed a correlation to the pleural microbiome, which was associated with an exudative composition of the pleural effusion.

Conclusions:
Lung transplantation and time after lung transplantation seem to affect the pleural microbiome. As the pulmonary and pleural microbiome directly correlated, we suggest a communication of the microbiome of both compartments.

Abstract No. 610

Effect of AAT augmentation therapy on Bronchiolitis Obliterans (BO) development in mice model

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Background: Obliterative bronchiolitis (BO) is one of the major limitations to long-term survival after lung transplantation (LuTx), which is recognized as a major factor of chronic lung allograft dysfunction (CLAD). Acute rejection is a significant risk factor that leads to BO development. Therefore, therapeutic strategies limiting the severity of acute inflammation can be of critical importance preventing acute rejection episodes and BO. Both preclinical and clinical experiences show that the therapy with plasma purified alpha1-antitrypsin (AAT) is beneficial for a broad spectrum of inflammatory conditions. We previously demonstrated that therapy with AAT suppresses the acute rejection after LuTx in a mouse model.
Methods: Herein, we aimed to investigate if prophylactic therapy with AAT prevents BO development in an orthotopic single LuTx model (C57BL/10 as donors and C57BL/6 as recipients) mice model. Transplanted mice were treated with cyclosporine (15 mg/kg) alone in the first week every day or with cyclosporine administration combined with AAT therapy (5 mg/kg) twice a week until the sacrifice 8 weeks after the LuTx.
Results: As illustrated in figure 1, mice not receiving AAT therapy (controls, n=5) develop BO and lung rejection whereas those receiving AAT therapy (n=3) did not develop BO and show only mild acute rejection.
Conclusions: The beneficial effects of AAT seem to involve anti-protease and immunomodulatory activities of AAT. AAT therapy may lower inflammatory responces due to ischemic injury or infections, and prevent risk of developing BO, which is one of the chronic LuTx rejection.

Abstract No. 611

Generative Lung Architecture Modeling (GLAM) by 3D image Generation and Rendering

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Drug development campaigns often rely upon model organisms to serve as a proxy for human subjects. However, animal models are often non-predictive and produce misleading results which cannot be translated into humans. Our goal is to develop machine learning-based methods for complex, 3D printable biological tissues to create new generative models of “humanized” disease tissue to address the limited availability of human and animal tissue. The resulting 3D prints will serve as standardized biological surrogates for pre-clinical high-throughput drug screenings, and ultimately as scalable functional lung substitutes. High resolution 3D microscopy was used to generate datasets of lung tissue architecture. Tissue was taken from mouse (healthy, fibrotic, and emphysematous) and human lungs. Tissue was processed into precision cut lung slices (PCLS) or preserved in larger pieces (whole lobes). Whole lobes were cleared using a simple ultrafast multicolor immunolabelling and clearing approach (SUMIC). The tissues were stained for extracellular matrix (ECM) and imaged using confocal or light sheet microscopes. The datasets were used for segmentation, computational modelling and 3D mesh generation. The data was segmented using the Napari plugin for accelerated pixel and object classification (APOC) as ground truth annotation and trained with a random forest classifier, for each condition. Reviews indicate the possibility to separate lung tissue conditions based on morphological similarity and will be further assessed for their potential. A software solution for generating printable meshes from the segmentations was deployed and will be adjusted to match the requirements of the 3D printing. 3D printing from these 3D mesh structures was accomplished by two-photon stereolithography.

Abstract No. 612

Towards the development of the Biohybrid Lung: Endothelialization of a left ventricular assist device with porcine endothelial cells for integration and testing in a porcine in vivo prototype

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Introduction
Along the development of the biohybrid lung (BHL), integration of a left ventricular assist device (LVAD) may ensure efficient blood transport through the future BHL oxygenator. Following the same strategy as for the BHL, establishment of an endothelial cell (EC) monolayer on all blood-contacting surfaces of the LVAD is hypothesized to reduce thrombotic complications. While endothelialization of individual impeller, housing and sintered inflow cannulas could be achieved with human ECs, we now aimed at endothelialization of the complete device with porcine ECs, allowing future testing in vivo.

Methods
ECs from porcine aortas (pAECs) were isolated and characterized for phenotype and proliferative capacity. After seeding on different original or 3D-printed and fibronectin (FN) coated LVAD surfaces, EC viability and functioning were assessed. Expression analysis of relevant genes was carried out to assess EC behavior on different materials and on the impeller, before and after medium flow during impeller rotation (<1000 rpm) for 24h.

Results
pAECs were successfully isolated and expanded, while remaining positive for CD31 (>90%). The specific surfaces of the LVAD (impeller, housing and inflow cannula), as well as 3D-printed housing parts, were entirely and confluent endothelialized when FN coated. Gene expression analysis revealed that the ECs kept their non-inflammatory /non-coagulative status on the different surfaces. Under rotation, the vast majority of the ECs remained on the impeller surface and maintained a physiological non-activated gene expression.

Conclusion
The blood-contacting LVAD surfaces were successfully endothelialized with pAECs, and are now ready for further testing under conditions closer to the clinical scenario, potentially in a pig model.

Abstract No. 613

"Super Survivor" after Lung Transplantation – Protective Role of Macrophages?

Edith Katharina Plucinski[1], Jan-Christopher Kamp[2], Jens Gottlieb[2], Tobias Welte[2], Marius Hoeper[2], Hans-Heinrich Kreipe[1], Danny Jonigk[3], Christopher Werlein[1], Lavinia Neubert[1], Mark Philipp Kuehnel[3]

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Lung transplantation (LTx) is often the last remaining therapeutic option for patients with end-stage lung diseases, such as idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease, or pulmonary hypertension. Despite numerous advances in the field of transplant surgery and follow-up care, the mean graft survival after LTx remains significantly limited at about 7 years. Various complications such as cellular or humoral rejection still challenge clinicians and recipients. Nevertheless, we could identify a group of patients that live for years without any complication after LTx, termed "super survivors". On the histological level, this group is characterized by a high number of alveolar macrophages despite the lack of clinical signs of infection or pulmonary remodelling. To investigate the role of the innate immune system with macrophages as major players in graft tolerance, we analyzed lung tissue from transbronchial biopsies. Therefore a whole transcriptome analysis of alveolar macrophages in n=16 super survivors as well as n=12 healthy controls was performed using the novel Nanostring GeoMx spatial transcriptomics technology. We identified a specific gene expression pattern in alveolar macrophages from LTx super survivors, distinct from the classical M1-M2 scheme. Here, we found macrophages exhibiting characteristics of anti-arteriosclerotic and anti-inflammatory macrophages in the context of long-term survival after LTx. We speculate that these macrophages have a protective effect on the allograft.

Abstract No. 614

A model to study the effects of fibrotic ECM in neoplastic and interstitial lung diseases on fibroblasts

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Fibrotic remodeling is a key feature in interstitial lung diseases and a baneful emergence in neoplastic lung diseases. In both cases, it worsens the disease state and impedes adequate therapy. Fibroblasts are the main producers of extracellular matrix (ECM). In studies using decellularized ECM from IPF patients, it could be shown that quiescent fibroblasts can be activated upon contact with excessive amounts of stiff extracellular matrix. Those hyper-activated fibroblasts deposit even more extracellular matrix, which can cause a vicious circle of fibroblasts indirectly activating more fibroblasts, leading to a continuous progression of fibrotic remodeling. In our ongoing study, we aim to investigate this aforementioned phenomenon in a double tracked approach, investigating the *in vivo* situation as well as establishing an *in vitro* model. On the one hand, we use a new spatial transcriptomics platform to specifically target fibroblasts in the stromata of different fibrotic neoplastic and interstitial lung diseases and compare their transcriptome among each other as well as with the transcriptome of fibroblasts from healthy lungs. On the other hand, we are establishing an *in vitro* model by decellularizing lung tissue samples from different fibrotic neoplastic and interstitial lung diseases, thereby generating acellular lung scaffolds of extracellular matrix, which are used for seeding and incubation with healthy primary fibroblasts, isolated from healthy regions of lung resection tissue. In the end, we aim to identify similarities and differences between abnormally activated fibroblasts in various fibrosis-associated lung diseases and identify possible therapeutic targets in fibroblasts that might be beneficial in different fibrosis-associated lung diseases.

Abstract No. 615

Investigation of the influences of human transgenes on blood-endothelial cell interaction in pulmonary xenotransplantation.

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Due to the shortage of transplantable human organs, current research is focusing more on possible alternatives such as porcine organ xenotransplantation. For hearts (945/195 (life-supporting) days), and kidneys (499 days), organ survival in primates has been greatly improved in recent years, and first groups have started with trials in human (brain-dead) patients. However, there are still some difficulties, especially with other organs such as lungs. In primates, survival is still less than one month after xeno-lung transplantation [1]. One idea to solve not only species-specific but also organ-specific incompatibilities is to use gene knockouts (GGTA1, B4GALNT2, CMAH) to reduce the organ immunogenicity and human transgenes (e.g. hCD47, CD55) for the immunosuppression in the recipient. In this context, the overall aim of this project is to identify human transgenes and gene knockouts that have a positive impact on tolerance in porcine-human lung transplantation. For this purpose, this new project is divided into three parts: First, porcine lung endothelial cells (pLEC) will be isolated and immortalized to obtain cell lines (ipLEC). Second, these ipLECs will serve as the basis for an *in vitro* model using a BioFlux microfluidic chamber and human blood or blood products. Lastly, some gene knockouts and human transgenes (knockins) will be integrated into these ipLECs to investigate *in vitro* the lung-specific effects of these genes in the context of coagulation and ipLEC activation. In the first part of the newly started project, different isolation methods (e.g. tissue digestion, lung perfusion) and cultivation conditions for pLEC from microvessels and/or larger vessels are now being tested and compared.

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Abstract No. 616

Towards the development of the biohybrid lung: Analysis of endothelial cells residing on gas exchange membranes under hyperoxic workload conditions

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Background
The implantable biohybrid lung (BHL) is under development for providing an alternative to lung transplantation, the only curative treatment option for patients with end-stage lung disease so far. On the basis of gas exchange hollow fiber membrane (HFM) technology used in clinically applied extracorporeal membrane oxygenation (ECMO) the polymethylpentene (PMP) HFM and all blood-contacting surfaces of the BHL will be covered with endothelial cells (EC) to prevent thrombotic occlusion of the device and to allow long-term use. ECs will be located between the oxygen-rich gas inside the HFMs and the hypercapnic/hypoxic blood of the patient, causing a non-physiological oxygen gradient. This environment was imitated in vitro to assess EC perseverance.

Methods
PMP films were coated with fibronectin and endothelialized. Upon adaption to disease related blood gas levels, ECs were exposed to hyperoxic (95% pO_2) conditions for 24h. After exposure ECs were assessed for change in viability, apoptosis and accumulation of reactive oxygen species (ROS) using flow cytometry (FACS). Expression level of oxidative stress- or inflammatory related genes was measured via qRT PCR. A 3D-printed model oxygenator was commissioned that allowed assessment of ECs seeded on HFM under ventilation and medium perfusion..

Results
FACS analyses revealed that ECs tolerated the hyperoxic conditions. The EC populations remained viable (normoxia: 86.56% vs hyperoxia: 86.73%), while no elevated numbers of ROS accumulating ECs were detected (normoxia: 6.09% ROS^+ vs hyperoxia: 1.53% ROS^+). Also, upregulation of prothrombotic/proinflammatory genes was not detected.
Conclusion
The data suggests that the tested ECs were able to tolerate the workload conditions potentially existing inside the BHL for at least 24h.

Abstract No. 617

Utility of GR-Scale in lung transplant recipients

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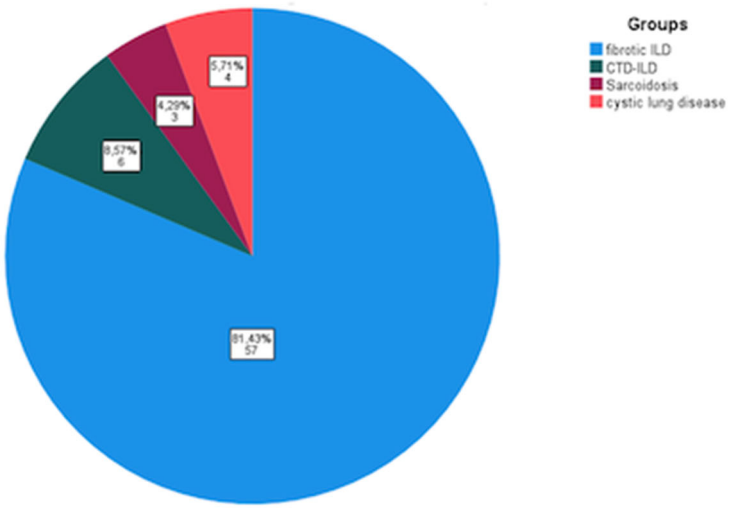
Background: Scallan et al. [1] proposed the R-Scale as a new questionnaire to assess the health status in patients with IPF.
Objective: We aimed to investigate the utility of a German version of the R-Scale (GR-Scale) by analyzing its relationship to lung function parameters in lung transplant recipients.
Methods: 70 double lung transplant recipients from four different diagnostic ILD groups (see figure) completed the GR-Scale questionnaire. Pearson correlation coefficients were calculated between total scores and lung function parameters (PaO2, RV%TLC, FVC, FEV1, Tiffeneau index). Besides, the mean of the 5 single items and the total score of the GR-Scale were compared (t test) between patients with BOS 0 (n=57) and BOS 1 (n=7).
Results: Within the entire study population, the GR-Scale total score showed inverse correlations with PaO2 (p=0.033) and FEV1 (p=0.017), while trends towards a negative correlation for FVC and Tiffeneau index and towards a positive correlation for RV%TLC were found. In the four groups similar trends existed, which were not significant due to sample sizes.
For all single items and the total score numerically higher values were found in patients with BOS 1 as compared to BOS 0. Due to sample size these results were not significant.
Conclusion: Our prelimiting results suggest that GR-Scale reflects the functional status of lung transplant recipients and may aid clinical judgement of this patient population.



Pie chart of the 4 groups (entire study population n=70)
Group 1 (n=57): fibrotic ILD (IPF, NSIP cHP, uILD)
Group 2 (n=6): CTD-ILD
Group 3 (n=3): Sarcoidosis
Group 4 (n=4): cystic lung disease (CPFE, LAM)

References:
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Abstract No. 618

Establishment of preclinical xenogeneic lung transplantation analyses using an ex vivo perfusion system

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The application of genetic engineering in donor animals and new drug treatments has brought out remarkable progress in the field of xenotransplantation. It is shown that the whole procedure has to be adapted for each organ system eg.,for kidneys, we look into the erythropoietin and renin structure dissimilarity between porcine donors and recipients which causes anemia and hyperaldosteronism and for the heart, we look into higher blood pressures in NHP/human recipient resulting in xenograft hypertrophy [1]. The lung being one of the most volatile organs in terms of immune responses remains to be a huge barrier for xenotransplantation. Ischemic reperfusion injury (IRI) is the tissue damage caused when blood supply returns to the tissue. IRI leads to various stages of graft rejection and subsequent organ failure. The initial step towards porcine lung xenotransplantation is to be able to perfuse porcine lungs with human blood and sustain them in an *ex vivo* lung perfusion system. This project mainly focuses on the molecular aspects of cellular incompatibilities between porcine lungs and human monocytes, comprising of 3 phases:
Analyze whether human cytokine and pig cytokine exposure bring about similar differentiation patterns and functions in human monocytes.
Investigating the dual function of porcine surfactant protein D in the presence of human leukocytes.
Understanding the cellular cross talk between human alveolar macrophages and porcine alveolar epithelial cells.
As this is a new project, I aim to achieve these objectives though *in vitro* experiments such as primary cell culture, biochemical analysis for surfactant isolation, Immunohistochemical analysis, FACs and protein analysis with Western blotting and ELISA techniques.

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Abstract No. 619

Smoking induced trained-immunity of neutrophils contributes to Lung Transplant Rejection.

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Cigarette smoking (CS) is a major risk factor for chronic obstructive pulmonary disease (COPD), with lung transplantation the only remaining end-stage treatment option for COPD patients. However, early and late complications after transplantation continue to be a major cause of morbidity and mortality globally. Recent research shows that neutrophil infiltration into the graft contributes to an increased risk of acute rejection. However, the precise mechanism underlying susceptibility amongst individuals remains unknown. Therefore, we hypothesize that CS induced epigenetic regulation of neutrophils induces a trained immune phenotype sensitizing to graft rejection. Our initial findings reveal that bone marrow isolated neutrophils from mice exposed to both acute (3d) and chronic (2month) CS demonstrated extended neutrophil lifespan, increased neutrophil elastase (NE) and myeloperoxidase (MPO) expression with increased NET formation. Importantly, a subsequent pro-inflammatory hit of LPS mimicking secondary graft challenge further enhanced these parameters and up-regulated expression of IL-1β and the pro-inflammatory immunomodulatory long non-coding RNA Neat1 compared to LPS treated neutrophils that were isolated from filtered air exposed mice. Taken altogether, targeting CS-induced trained neutrophils may represent novel therapeutic avenues addressing lung transplant survival.

Abstract No. 620

Description of relevant pro-fibrotic signalling pathways and implementation of a microphysiological system in human ex vivo lung tissue slices

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Pulmonary fibrosis (PF) comprises a series of rapidly progressive lung diseases characterized by uncontrolled deposition of extracellular matrix (ECM), fibroblast proliferation and destruction of the cellular architecture. Current animal models often do not correspond to the exact clinical picture of the disease. The translational model of living human Precision Cut Lung Slices (PCLS) offers new opportunities to generate data of clinical relevance for the development of novel anti-fibrotic drugs.

PCLS prepared from non-fibrotic tissue sections triggered with a pro-fibrotic cytokine cocktail (TGF-β/TNF-α) reveal elevated RNA level of several integrins (ITGAV or ITGB6), ECM remodeling enzymes (e.g. MMP13) as well as cytokines (VEGF, IL-1β, IL6). Supernatants of PCLS prepared from PF patients reveal higher levels of FN1 (2-fold) and pCol1a1 (5-fold). Gene set enrichment study identified 5 significantly distinct genetic patterns in non-fibrotic vs. fibrotic PCLS, including molecular pathways of p53- and FoxO signaling upon others.

We further, improved the model for cultivation in a microphysiological system (MPS) to investigate (1) the cultivability of the slices and (2) identification of relevant pathomechanisms in a physiological setting. Preliminary results indicate higher viability and higher metabolic activity in MPS cultivated PCLS as well as similar cytokine profiles as compared to the regular culture. Most importantly, in both experimental set-ups, the efficacy of an anti-fibrotic reference treatment could be confirmed.

Overall, PCLS represent a highly translational model to study underlying mechanisms of early-onset- or end-stage-fibrosis. The use of MPS allows an optimized and extended cultivation of fibrotic PCLS to include later time points for the drug testing.

DA Lung Cancer: Abstract No. 701 – 748

Abstract No. 701

Specific signals from Influenza infection induce tumor cell killing by orchestrating macrophage phenotype

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Macrophages have pivotal functions in homeostasis and many physiological processes beyond innate immunity. M1 macrophages are pro-inflammatory and tumor-inhibiting macrophages, while anti-inflammatory M2 macrophages are associated with tissue repair and tumor growth. During the course of diseases such as lung cancer or infections, alveolar niche can shape different macrophage phenotypes towards M1 or M2. The basis of molecular regulation of this polarization imbalance remains unclear, because the environmental cues in the local niche that drive tissue macrophage polarization *in vivo* are unknown. Therefore in this study we wanted to focus on macrophage plasticity in tumor-infection niche, and we hypothesized that macrophage plasticity can be targeted by infection cues to activate a tumor-inhibiting phenotype in lung cancer. By incubating tumor-promoting macrophages with Influenza A infection (IV) BALF, we found that d7 IV BALF reprogrammed macrophages towards a pro-inflammatory state and it also induced apoptosis of mouse lung cancer cells. In order to investigate which secretory factors drive this reprogramming, we conducted mass spectrometry analysis of d7 IV BALF. By secretome analysis, we detected SAA4 is significantly regulated in d7 IV BALF. We incubated tumor-promoting macrophages with SAA4 recombinant protein to assess its effect on the phenotypic shift and we showed that SAA4 induced a tumor-inhibiting phenotype. Furthermore, to unveil the transcriptomic signature of reprogrammed macrophages, we performed RNA-sequencing and identified transcription factors and upregulated genes to investigate their effects in reprogramming. Additionally, we used lung organoids and tumor precision cut lung slices and showed tumor cell inhibition by regulated macrophage phenotype.

Abstract No. 702

Peripheral inflammation featuring eosinophilia and/or neutrophilia impacts the survival and infiltration of eosinophils within the tumor among different histological subgroups of NSCLC patients.

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Background: Compelling evidence supports the connection between comorbidities and survival rates in NSCLC patients. Sensitization and subclinical inflammation can vary among the different histological subgroups of NSCLC patients. Blood eosinophils and/or neutrophils levels and eosinophils infiltration in tumor regions may influence the survival rates of NSCLC patients.
Methods: A retrospective study „Lung cancer cohort“ including 3,143 patients with NSCLC was conducted, the data was collected from the Data Warehouse of the German Center for Lung Research (DZL) and Lungbiobank at Thoraxklinik Heidelberg between 2010 and 2020. The impact of blood eosinophilia and /or neutrophilia on the survival rates was studied in this cohort. Sensitization to food and/or inhalant (sIgE), and hs-CRP was assessed in a sub-cohort including 212 NSCLC patients. Finally, the tumor infiltration of eosinophil cells was estimated in another sub-cohort of 60 NSCLC patients.
Results: Our study revealed that the sensitization to at least one food or inhalant allergen (sIgE) is higher in adeno-LC compared to non-adeno-LC. In contrast, hs-CRP was higher in the non-adeno-LC versus Adeno-LC. The study found that eosinophilia and/or neutrophilia negatively impacts survival outcomes for NSCLC patients with a clear difference between histological subgroups. Additionally, blood eosinophilia can cause a significant infiltration of eosinophils into the peritumoral region of the lung tissue.
Conclusion: This study pointed out the important role of eosinophilia and/or neutrophilia on eosinophilic infiltration intensity in TME and the survival rate of NSCLC patients with different patterns between adeno-LC and non-adeno-LC. This may suggest the important role of eosinophils in shaping the TME

Abstract No. 703

Integrated circulating tumor DNA and cytokine analysis for therapy monitoring of ALK-rearranged lung adenocarcinoma

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Approximately 3-7% of non-small cell lung cancers harbor a structural rearrangement in the *ALK* gene (ALK+ NSCLC) resulting in the constitutive activation of the protein. ALK inhibitors have improved the prognosis of ALK+ NSCLC patients, however, treatment resistance eventually develops. Close monitoring of therapy success or failure is critical for patient management. Detection of circulating tumor DNA (ctDNA) in biological fluids is a minimally invasive alternative to tissue biopsy for therapy monitoring. Cytokines are released in the tumor microenvironment to influence inflammation and tumorigenic mechanisms, and serum cytokine levels have been associated with the risk for tumorigenesis in various cancers. We investigated the biomarker utility of circulating cytokines *vis-à-vis* ctDNA in ALK+ NSCLC patients and explored the optimal combination of molecular parameters that could indicate disease progression. Longitudinal serum samples (n=296) were collected from patients (n=38) under tyrosine kinase inhibitor therapy, and assayed to quantify IFN-γ, IL-1β, IL-6, IL-8, IL-10, IL-12p70, MCP1, and TNF-α. Serum IL-6, IL-8 and IL-10 were elevated at progressive disease, with IL-8 having the most significant impact as a biomarker. Integrating changes in IL-8 with ctDNA parameters maximized the performance of the classifiers in identifying disease progression, but this did not significantly outperform the model based on ctDNA alone. Further validation in a larger and prospective cohort is necessary to determine whether the addition of cytokine evaluation could improve current tumor monitoring modalities in the clinical setting.

Abstract No. 704

CD8+ T cell subpopulation (Tc17) Induce a metabolic enriched tumor microenvironment enabling lung cancer progression

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CD8+ T cells are key mediators of antitumor function that specifically recognize and react to tumor-expressing antigens. However, they are not as effective as expected, this dysfunctional state of CD8+ T cell subpopulations can be linked to impaired metabolic pathways different from that of cytotoxic functional effector T cells (CTLs). We, therefore, aim to understand the unraveled role of IL-17-producing CD8+ T cell subpopulations (Tc17) in lung cancer. Our findings uncovered increased infiltration of Tc17 (IL17+CD8+) T cells in lung tumor tissues compared to lung healthy tissues. Conditioned medium from Tc17 cell alone and Tc17 co-cultures with tumor cells displayed enhanced tumor cell proliferation and migration in vitro. Notably, the double knockout of IL17 A+F mouse isolated Tc17 cell conditioned medium and co-cultures with tumor cells restores CD8+ T cells tumor-inhibiting effects. Furthermore, metabolomics profiling of conditioned medium from wild type and IL17A+F DKO mice Tc17 cells indicate the explicit metabolic profile, most notably the TCA cycle, and metabolites including malate, aspartate, and pyruvate which are downregulated in the conditioned medium of IL17 A+F DKO Tc17 cells. Furthermore, Tc17 conditioned medium activates the naive macrophages towards tumor-promoting macrophages by increasing M2 phenotypic markers (IL-10, Arginase) inducing tumor cell proliferation and migration. In conclusion, we identified Tc17 as a novel pro-tumorigenic CD8+ T-cell subtype in lung cancer, which enables tumor growth. We also uncovered the intrinsic regulatory role of IL-17 in CD8+ T cells metabolism, a loophole in CD8+ T cell antitumor function promoting lung cancer progression, which results in a poor prognosis for patients.

Abstract No. 705

''Do not eat me'' LILRB4 signaling as a novel macrophage immunotherapy in lung cancer

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Lung cancer is the main cause of cancer-related deaths. Although T cell-targeting immunotherapies improved patient survival, the effectiveness is still low. Today macrophage-mediated immune escape via upregulation of ''Do not eat me'' signals (e.g SIRPa, Siglec10) is coming in light. The leukocyte immunoglobulin-like receptors (LILRs) are the emerging targets for cancer immunotherapy, targeting tumor-associated macrophages (TAMs). An extensive expressional profiling of LILRs in various experimental macrophage models revealed that, among LILRs, LILRB4 is highly expressed in TAMs. Also, double immunofluorescent staining in paraffin sections of human lung tumor biopsies confirmed the increased expression of LILRB4 in CD68+ macrophages. Interestingly, genetic ablation of LILRB4 in *in vitro* TAMs reduced the expression of tumor-promoting M2 macrophage genes (CSF1R, ALOX15B). At functional level, macrophage-specific LILRB4 silencing does not affect the proliferation and apoptosis of cancer cells. However, cancer cells' phagocytosis significantly increased upon macrophage-specific genetic ablation and antibody-mediated blockade of LILRB4. RNA sequencing showed differential gene (DEGs) regulation in TAMs_LILRB4_KD compared to TAMs. Pathway analysis from these DEGs revealed that LILRB4 knockdown inhibits cell cycle progression. This result was further confirmed by flow cytometry, which correlates with decreased phagocytic activity in the G2/M phase. Moreover, a synergetic effect was observed upon simultaneous blockage of LILRB4 and EGFR, but not with other immunotherapeutic antibodies (e.g a-PDL1, a-PD1). Taken together, our data suggest that LILRB4 could play a role in lung cancer macrophage-specific immune evasion and could be a promising target for lung cancer immunotherapy.

Abstract No. 706

Pericentromeric satellite lncRNAs regulate pro-tumorigenic functions of cancer-associated fibroblasts in lung tumorigenesis.

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The abnormal tumor microenvironment (TME) often dictates the therapeutic response of cancer to chemo- and immuno-therapy. Aberrant expression of pericentromeric satellite repeats has been reported for epithelial cancers, including lung cancer. However, the transcription of tandemly repetitive elements in stromal cells of the TME has been unappreciated, limiting the optimal use of satellite transcripts as biomarkers or anti-cancer targets. We found that transcription of pericentromeric satellite DNA (satDNA) in mouse and human lung adenocarcinoma was observed in cancer-associated fibroblasts (CAFs). In vivo, lung fibroblasts expressed pericentromeric satellite repeats HS2/HS3 specifically in tumors. In vitro, transcription of satDNA was induced in lung fibroblasts in response to TGFβ, IL1α, matrix stiffness, direct contact with tumor cells and treatment with chemotherapeutic drugs. Single-cell transcriptome analysis of human lung adenocarcinoma confirmed that CAFs were the cell type with the highest number of satellite transcripts. Human HS2/HS3 pericentromeric transcripts were detected in the nucleus, cytoplasm, extracellularly and co-localized with extracellular vesicles in situ in human biopsies and activated fibroblasts in vitro. The transcripts were transmitted into recipient cells and entered their nuclei. Knock-down of satellite transcripts in human lung fibroblasts blocked the formation of an inflammatory CAFs phenotype which resulted in the inhibition of their pro-tumorigenic functions. In sum, our data suggest that satellite long non-coding (lnc) RNAs are induced in CAFs, regulate expression of inflammatory genes and can be secreted from the cells, which potentially might present a new element of cell-cell communication in the TME.

Abstract No. 707

Pulmonary hypertension in cancer

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Background: Cancer remains a prominent contributor to global mortality rates and the presence of cardiopulmonary comorbidities can significantly compromise cancer prognosis. Recently, our research group has identified pulmonary hypertension (PH) as a novel form of comorbidity associated with lung cancer. In this study, we aimed to investigate the incidence and impact of PH in cancers beyond lung cancer and compare the incidence and impact of PH on survival between these two groups.
Methods: We retrospectively analyzed 670 lung cancer patients and 1030 patients with solid tumors other than lung cancer, including colorectal, head and neck, urological, and central nervous system tumors. We measured the pulmonary artery to ascending aorta diameter (PA/A) ratio from baseline computed tomography images to identify patients with a PA/A ratio >1 as a surrogate marker for PH.
Results: The incidence of PH in the lung cancer cohort was 22.5%, while the incidence in the cohort of other solid tumors was 10.8%. Patients with a PA/A ratio >1 had significantly reduced overall survival in both cohorts (207 days vs. 568 days in lung cancer patients and 880 vs. 1370 days in other solid tumor patients). There was no correlation between lung metastasis and PH, and a subgroup analysis showed that PH was associated with decreased lung and heart function but not with emphysema.
Conclusion: Our study demonstrates that PH is less prevalent in solid tumors other than lung cancer, but still influences prognosis. However, the impact of PH on survival is less pronounced compared to lung cancer associated PH, indicating a distinctiveness of this novel PH form. Our findings also emphasize the importance of further studies in the field of cardio-oncology.

Contribution of a Disease-spanning Working Group: Lung Environment Interaction

Abstract No. 708

The impact of PD-L1 expression and co-mutations on outcome of KRAS-driven non-small-cell lung cancer

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Background
Interest has surged for *KRAS*^{mut} NSCLC after approval of sotorasib, but data on the role of co-mutations and benefit from chemo(CHT)-immunotherapy (IO) remain scant.
Methods
We analyzed patients with *KRAS*^{mut} NSCLC in the Thoraxklinik from 01/14 until 01/21.
Results
No differences were observed between *KRAS*^{G12C} (n=163) and *KRAS*$^{non\text{-}G12C}$ (n=207) regarding clinicopathological features and OS (median 19.4 months). However, there was a strong association of PD-L1 TPS with OS (20.2 *vs.* 9.7 months for PD-L1^{high} vs. PD-L1^{neg}, p=0.011) and PFS (5.7 *vs.* 1.9 months, p=0.004) under IO. Within the PD-L1^{neg} subset, OS was longer for *KRAS*^{G12C} compared to *KRAS*^{other} (22.6 *vs.* 12.1 months, p=0.032). This was driven by the longer PFS under CHT-IO (9.7 *vs.* 4.5 months for *KRAS*^{G12C} *vs.* *KRAS*^{other}, p=0.005), particularly in the absence of *TP53*^{mut} (8.5 vs. 4.0 months with p=0.004 for *TP53*^{wt} patients; p>0.50 for *TP53*^{mut}) and *KEAP1*^{mut} (11.3 *vs.* 5.1 months with p=0.01 for *KEAP1*^{wt} patients; p>0.70 for *KEAP1*^{mut}). Also, across all patients, *KEAP1*^{mut} showed a trend for shorter PFS under CHT-IO (2.7 vs. 8.0 months, p=0.07) and IO (1.9 vs. 3.9 months, p=0.081) and shorter OS (15.9 *vs.* 19.5 months, p=0.068) compared to *KEAP1*^{wt}, in contrast to *STK11*^{mut} (p=0.42-0.92 for the same comparisons). *KRAS*^{mut} NSCLC after chemotherapy and immunotherapy showed poor prognosis with a median PFS of 3 months, and a median OS of 6.8 months.
Conclusions
In PD-L1^{neg} NSCLC, *KRAS*^{G12C} is associated with better outcome under CHT-IO and longer OS compared to *KRAS*^{other}, especially in the absence of *TP53* and *KEAP1* co-mutations. *KEAP1*, but not *STK11* mutations are associated with impaired benefit from (CHT-)IO in *KRAS*^{mut} NSCLC. Treatment of *KRAS*^{mut} NSCLC after CHT and IO represents an unmet medical need.

Abstract No. 709

Durvalumab consolidation after sequential chemoradiotherapy prolongs survival in locally advanced non-small cell lung cancer – a retrospective propensity score analysis

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Durvalumab consolidation after definitive chemoradiotherapy (CRT) is now standard care for patients with locally advanced non-small cell lung cancer (NSCLC) based on the PACIFIC trial that investigated durvalumab therapy after concurrent CRT (conCRT). However, information, in particular real-world data, about the benefit of durvalumab in patients who beforehand have received sequential CRT (seqCRT) is scarce.
In this retrospective, single-center study, we analyzed stage III NSCLC patients receiving durvalumab consolidation therapy following either conCRT (n=17) or seqCRT (n=31). No differences were found between the two groups regarding therapy duration, side effects, or outcome. For a direct comparison of real-life efficacy of durvalumab consolidation after seqCRT with a control group not treated with immunotherapy, propensity score matching (PSM) was employed to define an appropriate control group out of a historic cohort of NSCLC stage III patients treated with seqCRT without durvalumab consolidation. Comparison of outcome in the PSM-defined control group (n=76) with durvalumab-treated patients (n=31) revealed a significant improvement for patients who received the consolidation therapy after seqCRT (median progression-free survival [PFS] 15.3 months [95% CI, 11.9 months to not reached] vs. 12.8 months in the control group [95% CI, 10.5 to 15.4 months], Hazard Ratio [HR] [95%CI]: 0.472 [0.262, 0.853], p=0.0129, and 2-year PFS rate 42.3% vs 23.6%). 2-year overall survival rates were 63.3% in the durvalumab- and 48.3% in the control group (HR: 0.502 [0.285, 0.883], p=0.0169).
These findings provide real-world evidence for the efficacy of durvalumab consolidation therapy in patients with locally advanced NSCLC with prior sequential CRT.

Abstract No. 710

Cross linking TGFβ signaling and metabolism in lung adenocarcinoma

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The pleiotropic cytokine transforming growth factor beta (TGFβ) is known to play a dual role in the context of cancer: while TGF-β functions as a tumor suppressor in premalignant cells, it drives cancer progression in later stages by inducing epithelial-to-mesenchymal transition (EMT). As EMT and migration are highly energy consuming processes, ATP levels are increased during TGF-β-induced EMT, suggesting a close relation between TGFβ signaling and metabolism. However, the underlying mechanisms remain to be resolved.
Potential candidates as connecting element are high-energy metabolic messengers such as inositol pyrophosphates (PP-InsPs), since it has been shown recently that PP-InsPs are required for tumor progression and metastasis in cell and mouse models. First evidence points to a direct interaction between PP-InsPs and TGFβ signal transduction through the stabilization of SMAD-complexes, potentially leading to alterations in gene expression. To address this hypothesis and examine the impact of PP-InsPs on SMAD complex phosphorylation, TGFβ target gene expression and invasive behavior, we modified the expression levels of Inositol Hexakisphosphate Kinase 1 (IP6K1) the central enzyme envolved in the generation of PP-InsPs and established a stable IP6K1 knock out as well as an IP6K1 overexpressing lung adenocarcinoma cell line H1975. Through a proteomics approach examining paired tumor and tumor-free samples of adenocarcinoma patients, we observed alterations in the abundance of TGFβ signaling components, glycolytic enzymes and a strong overall alteration in the abundance of proteins of the phosphoinositide metabolism, supporting an important link between TGFβ signaling and energy metabolism as well as the role of inositol phosphates in the development of lung cancer.

Abstract No. 711

Cancer-associated fibroblasts mediate drug resistance in ALK-rearranged lung adenocarcinoma cells by sustained lipogenesis

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Targeted therapy interventions using tyrosine kinase inhibitors (TKIs) provide compelling response rates in ALK+ lung adenocarcinoma patients. The occurrence of resistances, however, still poses a major clinical challenge. Several lines of evidence support a crucial role of cancer-associated fibroblasts (CAFs) to such treatment insensitivities, while underlying mechanisms remain poorly understood. We therefore aimed to uncover molecular networks shaping the therapeutic susceptibility of lung cancer cells towards ALK inhibition via tumor stromal cues in 3D co-culture settings.
Here, we show that CAFs were able to provoke resistance to ALK-TKIs in lung tumor spheroids as observed by reduced cell death and increased cell proliferation rates. Transcriptional differences between homo- and heterotypic tumor spheroids as interrogated by scRNA-sequencing revealed a CAF-driven increase of lipogenic targets (e.g. *FASN, SCD1, SREBF1)*, which was further validated *in vitro*. Heterocellular communication between the tumor and stromal cell compartment was examined through ligand-receptor-interaction analysis. Indeed, CAF-conditioned medium and *in silico* predicted CAF-secreted factors HGF and NRG1 were likewise able to mediate therapy resistance of ALK+ lung tumor spheroids via AKT signaling induction, concomitant with enhanced *de novo* lipogenesis and suppression of lipid peroxidation. Importantly, the established CAF-driven lipid metabolic-supportive niche of TKI-resistant lung tumor spheroids could be overcome by simultaneous targeting of ALK and SREBP-1. Together, our findings support a rationale of CAF modulated ALK-TKI resistance via lipid metabolic reprogramming and suggest a novel anti-neoplastic treatment strategy by targeting vulnerabilities downstream of oncogenic signaling.

Abstract No. 712

CD8+ T cell trajectory subtypes determine clinical outcome and therapy response in LUAD

Benteng Deng, Benteng Deng

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Cellular interactions and altered immune functions shape the tumor microenvironment and map clinical outcomes. Currently, the potential clinical significance of CD8+ T cell evolution in lung adenocarcinoma (LUAD) remains largely unexplored.
A total of 8,275 T cells and 1,149 LUAD patients were retrieved from eight independent public datasets. Seurat and Monocle algorithms were used to describe the evolution of T cells, and consensus clustering was further applied to identify molecular classifications. Subsequently, prognosis, biological characteristics, genomic variation, and immune landscape between different clusters were decoded. Most importantly, the cluster-based efficacy of immunotherapy and chemotherapy response was assessed.
Based on hub genes of CD8+ T cell trajectories, we identify two heterogeneous clusters (C1/C2), that determined clinical outcomes in the TCGA-LUAD cohort. C1 was significantly associated with a worse prognosis. Three independent cohorts validated the robustness and reproducibility of the two clusters. Further explorations clarified that C1 referred to a higher cell-proliferative LUAD, whereas C2 as an “immune-hot” with an elevated immune-inflammation performance. Using genomic variation analysis, C1 displayed the most conspicuous genomic instability. Assessment models of therapeutic intervention suggested that C1 were sensitive to conventional chemotherapy, while C2 were more responsive to immunotherapy.
In this study, based on CD8+ T cell trajectory/evolution we identified two clusters with different clinical outcomes, biological features, genomic signatures, immune landscape, and treatment responses. Altogether, this could facilitate individualized therapy to improve outcomes in LUAD.

Abstract No. 713

Comprehensive clinical profiling of a Lung squamous carcinoma cohort

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Worldwide, malignant tumors of the lung are the primary cause of cancer incidence and death, ranking as the highest tumor-related mortality. Lung squamous cell carcinoma (LUSC) is the second major subtype of non-small cell lung cancer, representing ca. 25% of all lung cancers still with limited treatment options. LUSC patients have poor clinical prognosis and lack targeted agents compared to LUAD. Therefore, there is an urgent need to understand LUSC clinical phenotypes and create real-life cohorts, to test biomarkers that predict progression of LUSC patients.
To address this need, we used the CPC BioArchive and the biobank of the Asklepios clinic Gauting to create a LUSC cohort. Our patient selection was conducted in accord with the LMU Ethics Committee and written informed consent was obtained from all patients. All patients between 2010-2016 with histologic LUSC diagnosis, surgically resected tumor tissue and/or matched blood were included. A total of 452 patients were initially selected. 149 had matched tumor tissue and preoperative serum samples. They were distributed among 118 male (79.2%) and 31 female (20.8%) patients with 81 smokers (54.5%), 64 ex-smokers (43.0%), and 3 never smoker (2.0%). 74 deaths (49.7%) were registered (31 reported as tumor-related(42.0%)). LUSC patients were staged according to IASLC/UICC7 (2010-2016). The stages were divided as followed: 58 patients with stage 1 (38.9%), 48 with stage 2 (32.2%), 37 with stage 3 (24.8%), and 6 with stage 6 (4.0%).
A comprehensive characterization of clinical features including age, gender, smoking status, lung function, radiologic, and molecular variables follows. This cohort will provide a valuable tool for future validation studies in a real-life setting, allowing cross-validation with other LUSC cohorts.

Abstract No. 714

The interplay between EGFR and MET receptor abundance and cross-talk determines the effectiveness of tyrosine kinase inhibitors in lung cancer patients

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Non-small cell lung cancer (NSCLC) is a major cause of cancer-related deaths worldwide. While first-generation tyrosine kinase inhibitor (TKI) treatments targeting epidermal growth factor receptor (EGFR) have been shown to extend survival for a few months, their effectiveness is temporary due to the emergence of therapy resistance. Although recent studies have identified the hepatocyte growth factor (HGF) receptor MET as a factor contributing to resistance to EGFR-TKIs, the underlying mechanisms are not yet fully understood.
We investigated signal transduction induced by EGF and HGF in four NSCLC cell lines with varying expression levels of EGFR and MET and carrying different EGFR mutations. Our findings suggested that co-stimulation of EGF and HGF activates MET phosphorylation in a cell type-specific manner. According to a dynamic pathway model, the ratio of EGFR and MET abundance regulates their cross-talk, which leads to an increased half-life of the MET receptor. Our hypothesis was confirmed by molecular biological approaches. We used mass spectrometry with data-independent acquisition (DIA) and targeted proteomics with parallel reaction monitoring (PRM) and established in an extended panel of NSCLC cell lines a wide variation in the receptor abundance ratio. By applying our proteomics methods to primary patient material, we confirmed a EGFR to MET surface expression ratio dependent effect on EGFR TKI treatment response and independence from the EGFR mutation status. The absolute quantification of the receptors by PRM, in combination with our mathematical model promises to facilitate the rapid stratification of lung cancer patients and identify those that are likely to benefit from treatment with novel TKIs or monoclonal antibodies targeting either EGFR, MET or both.

Abstract No. 715

Functional diversity of the TP53 mutome revealed by saturating CRISPR mutagenesis

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The tumor suppressor gene *TP53* is the most frequently mutated gene in various cancers. Unlike other tumor suppressors, *TP53* is mostly hit by missense mutations, of which more than 2,000 have been described in cancer patients. To take advantage of *TP53* mutation status for personalized therapy, a deeper knowledge of the functional ramifications of specific mutations is required as evidence of the functional heterogeneity of mutant p53 proteins mounts. Here, we report on a CRISPR-based saturation mutagenesis screen of 9,225 variants expressed from the endogenous *TP53* gene locus of a cancer cell. By tracking changes in the abundance of individual variants in response to specific p53-pathway stimulation, we were able to construct high-resolution functional activity maps of the *TP53* mutome, covering ~94.5% of all cancer-associated missense mutations. The results demonstrate the impact of individual mutations on tumor cell fitness with unprecedented precision and coverage, even revealing underlying mechanisms such as apoptosis. The high discriminatory power also resolves subtle loss-of-function phenotypes and highlights a subset of mutants as particularly promising targets for pharmacological reactivation. Moreover, the data offer intriguing insight into the role of aberrant splicing and nonsense-mediated mRNA decay in clearing truncated proteins due to not only nonsense, frameshift, and splice-site mutations but also missense and synonymous mutations.

Abstract No. 716

Treatment with low-molecular-weight heparin negatively effects lung cancer patient survival

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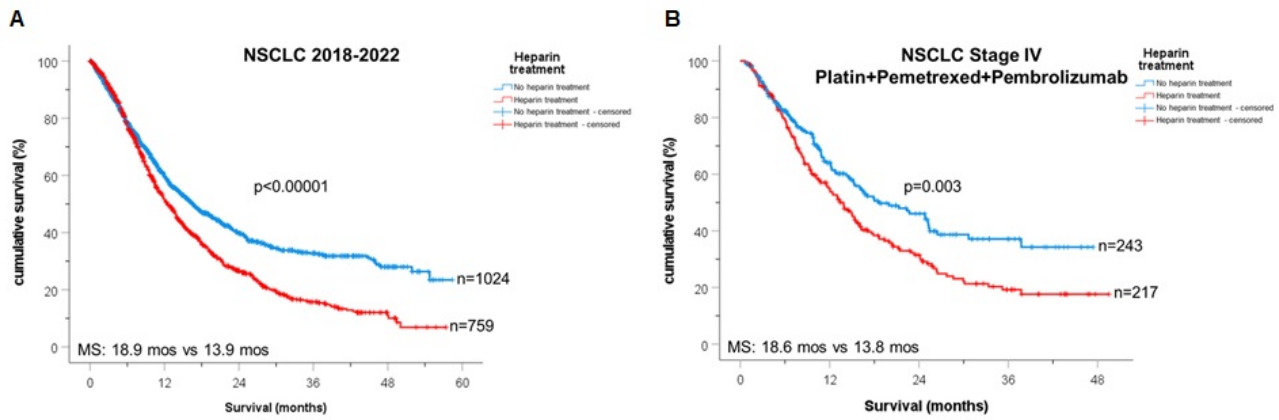
Hypercoagulable states and thromboembolism are well-known hallmarks of different malignancies, including lung cancer. Risk factors which lead to cancer-associated thrombosis include cancer type, radiotherapy, chemotherapy, and immunotherapy. To date, low-molecular-weight heparin (LMWH) preparations are administrated for prophylaxis and treatment of cancer-associated thrombosis. Pre-clinical findings indicate that, apart from the antithrombotic properties, LMWHs exhibit anti-angiogenetic and anti-metastatic effects. However, randomized trials have not been able to show survival advantages in lung cancer patients treated with LMWH. We aimed to assess the effects of LMWH therapy in a retrospective cohort of 2486 lung cancer patients included in the database of Heidelberg`s biobank.



Kaplan-Meier survival curves indicate reduced survival of patients receiving heparin therapy. (A) NSCLC patient overall survival. No heparin therapy: N = 1024, Heparin therapy: N = 759. (B) NSCLC stage IV patient survival with a combined therapy of Platin + Pemetrexed + Pembrolizumab. No heparin therapy: N = 243, Heparin therapy: N = 243.

Our data show that non-small cell lung cancer (NSCLC) patients in general or with stage IV cancer receiving LMWH therapy have significantly lower survival than those without LMWH therapy. Hence, LMWH therapy itself does not have beneficial effects on NSCLC patient survival and therefore should only be considered as a personalized therapy when definitely required.

Abstract No. 717

Unraveling Lung Cancer Mechanisms through Advanced Mass Spectrometry-based Proteomics

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Mass spectrometry (MS)-based proteomics provides a valuable opportunity for in-depth protein analysis of signaling events leading to lung cancer. We have established a low-input automated phosphoproteome pipeline, a pipeline for proteomics analysis of blood plasma and secretome, and a parallel reaction monitoring (PRM) panel to quantify different membrane receptors in the attomolar range or even the entire TGFb pathway.
To further advance our methods, we have implemented new MS acquisition methods, such as Data-independent acquisition (DIA), which showed higher proteome coverage than commonly used methods and allowed the quantification of low-abundance proteins. Using these MS-based methods, we have profiled different samples to advance our understanding of the mechanisms promoting lung cancer initiation, recurrence, and resistance to TKI. Specifically, we have analyzed immortalized lung cancer cell lines, tissue, and blood plasma from lung cancer patients.
Using our phosphoproteome pipeline, we estimated the degree of phosphorylation of key receptors implicated in lung cancer and observed their dynamics upon cytokine stimulation. Interestingly, the abundance of membrane receptors was highly heterogeneous among different cell lines, but downstream signaling pathway molecules remained relatively constant. This pattern was also observed in the tissue of lung cancer patients.
Our methods have shown significant separation between lung cancer patients and controls in plasma proteome. Additionally, evidence suggests significant protein pattern differences related to patients' gender. Our goal is to expand our understanding of disease-promoting mechanisms and identify potential targets for diagnosing and treating lung cancer.

Abstract No. 718

Robotic Assisted Bronchoscopy for Biopsy of Peripheral pulmonary lesions – first results from Europe

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Objective: Conventional bronchoscopy provides only limited approach to peripheral pulmonary lesions (PPL). The ION™ Endoluminal System is a shape-sensing robotic-assisted bronchoscope (RAB) that has already shown feasibility and is used in clinical practice in the US but not in Europe so far.
Methods: A prospective, single-center, single-arm study was conducted at the Thoraxklinik Heidelberg to investigate the clinical utility of RAB with ION™ for biopsy of PPL (1-3cm in largest dimension located at ≥4th generation airway). The primary outcome was rate of tool in nodule (TIN) as confirmed via intraprocedural computed tomography imaging with CIOS Spin. Secondary outcomes were procedural characteristics, diagnostic outcomes and adverse event (AE) rate.
Results: Twenty-three subjects with a median (IQR) nodule size of 20 (18,27)mm were included. Two procedures were stopped due to an endobronchial tumor or nodule resolution observed on imaging. Procedures were conducted by 2 interventionalists with a median (IQR) procedure time of 44 (30,60) min; procedure length decreased over time. Nodule visualization with radial EBUS was 86%; 76% of cases had ≤2 CT spins performed. TIN was obtained in 100% (21/21) of cases where biopsy was attempted; in one case TIN was confirmed in 2-dimensions with a diagnosis of carcinoma. Follow-up up to 6 months of subjects is ongoing; preliminary findings are: 12 malignant, 5 non-malignant, 4 non-diagnostic cases with no AE reported including no pneumothorax.
Conclusion: Preliminary results for the first European cases show shape-sensing robotic-assisted bronchoscopy is a safe procedure with promising results for enabling diagnosis of PPL.

Abstract No. 719

An in vitro model for studying SCLC development

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Small cell lung cancer (SCLC) is a highly aggressive cancer subtype that accounts for approximately 10-15% of all lung cancer cases. Despite significant advances in the treatment of other types of cancer, the prognosis for patients with advanced SCLC remains poor, with a five-year survival rate of less than 10%.There is a pressing need for research into the molecular mechanisms driving SCLC. However, the study of early carcinogenesis presents significant challenges because most patients are diagnosed with cancer in its advanced stages, making it difficult to obtain early tissue samples. In addition, the rarity of pulmonary neuroendocrine cells (PNEC), which are the primary precursor cells of SCLC and represent only ~0.01% of lung cells, limits the availability of material for in vitro experiments. To overcome this obstacle, we employ an in vitro differentiation model in which human induced pluripotent stem cells (hiPSCs) are differentiated into PNECs. We introduce tetracycline-inducible CRISPR/Cas9 into hiPSCs, allowing the inactivation of SCLC-associated tumor suppressor genes and investigation of the effects on PNEC expression profiles in a human cellular context. This approach is expected to provide valuable insights into early expression changes of PNECs and create a robust model for studying early carcinogenesis in SCLC.

Abstract No. 720

A toolbox for generation of genetically modified lung organoids

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Organoids are good in vitro models for studying the development and function of normal and diseased tissues. These three-dimensional miniature versions of human organs are generated upon directed differentiation of stem cells. Organoids can be derived from adult stem cells (ASC), isolated from donor tissues, or from pluripotent stem cells (PSCs). While ASC can be differentiated into only a limited set of cell types, defined by the ASC's tissue of origin, PSCs can be used for the generation of different cell lineages and production of different types of organoids. In our project, we are developing an iPSC-based platform for the safe, efficient, and fast generation of genetically modified human lung organoids. The platform consists of two key elements: a "genetic toolbox" for efficient cloning of vectors used in gene editing, and genetically engineered human iPSC lines with stable inducible expression of CRISPR components. In addition, these cell lines carry further modifications to improve gene editing efficiency, enable conditional expression or perform cell tracing. Our platform has a potential for generation of complex organoid models, creating more opportunities for replacing laboratory animals in basic and translational research.

Abstract No. 721

Longitudinal monitoring of cell-free DNA methylation in ALK-positive non-small cell lung cancer patients

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Background: DNA methylation (5-mC) signals in cell-free DNA (cfDNA) of cancer patients represent promising biomarkers for minimal-invasive tumor detection. The high abundance of 5-mC alterations in cancer permits sensitive assessment of multiple 5-mC biomarkers. Here, we performed 5-mC profiling in plasma of *ALK*+ non-small cell lung cancer (NSCLC) patients receiving tyrosine kinase inhibitor therapy.
Methods: Serial plasma samples (n=79) of 21 *ALK*+ patients and 13 healthy donors were collected alongside 15 *ALK*+ tumor tissue and 10 healthy lung tissue specimens. All plasma and tissue samples were analyzed by DNA methylation immunoprecipitation sequencing to generate genome-wide 5-mC profiles.
Results: We devised a strategy that identified tumor-specific 5-mC biomarkers by reducing 5-mC background signals derived from blood cells, followed by differential methylation analysis and biomarker validation using 5-mC profiles of *ALK*+ tumor tissues. The resulting 245 differentially methylated regions were enriched for NSCLC-specific 5-mC patterns in TCGA data and indicated transcriptional repression of several genes described to be silenced in NSCLC. Additionally, 5-mC-based tumor DNA quantification (5-mC score) was highly correlated to genomic alterations (Spearman, $\rho>0.6$) and associated to shorter overall survival (log-rank $p=0.025$). Longitudinal 5-mC scores reflected radiologic disease assessments and were significantly elevated at disease progression ($p=0.0023$).
Conclusion: We demonstrated a strategy to identify 5-mC biomarkers from plasma of cancer patients and integrated them to a quantitative measure of cancer 5-mC alterations. Using longitudinal plasma samples of ALK+ NSCLC patients, we highlighted the suitability of cfDNA methylation for prognosis and therapy monitoring.

Abstract No. 722

Integrated multi-omics unraveled a basal cell-dependent signature predictor of outcome and immunotherapy response in LUSC

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Lung squamous carcinoma (LUSC) is a major lung cancer killer with no biomarkers and limited therapeutic options. Although still unclear, transformation of the pseudostratified and basal epithelium is linked with its origin. With integrated transcriptomics, proteomics, genomics, epigenetics, and single-cell RNA sequencing (scRNA-seq), we explore basal cell-dependent signatures as therapy response predictors in LUSC.

Unsupervised clustering of the TCGA-LUSC cohort classified patients into "Hot" and "Cold" using immune and basal cell-associated hub genes. The "hot" cluster had higher immune scores, immune cell infiltration (ICI), and better immunotherapy response. The "cold" cluster had a higher proliferative basal cell enrichment score and higher expression of KRT5/6 and TP63.

Both clusters had a unique and distinctive mutational landscape (shared driver mutations: TP53, KRTAP-5-5; hot: NOTCH1, ZFR; cold: ARID1A, RECQL5). Copy number variants results showed that both clusters had shared amplifications and deletions, but also individual ones [amplification – hot: WHSC1L1, KAT6A; cold: EGFR, AKT1); deletion – hot: ROBO1, FHIT; cold: STK11)]. 15 key prognostic genes were identified, and for the first time, CTTNBP2 and COL22A1 predicted survival in 4 different LUSC cohorts.

Intercellular communication analysis of scRNA-seq showed a novel communication dependency between basal and immune cells that might influence immunotherapy response.

For the first time, our multi-omic integrative approach identified "Hot" and "Cold" clusters that differ substantially in their genetic landscape, a 15 key prognostic gene signature that reflect survival, and a basal cell-dependent communication that predicts immunotherapy response in LUSC.

Abstract No. 723

Whole transcriptome sequencing for the differentiation of non-pulmonary metastasis from primary NSCLC

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The correct diagnosis of a tumor is critical for clinical decision making and patient management. Incorporating immunohistochemistry and molecular pathology, histological techniques can lead to the correct diagnosis in the vast majority of cases. However, diagnostic borderline areas with a degree of uncertainty still exist, e.g., the differentiation between primary/second tumor/metastasis or the distinction between squamous cell carcinoma metastasis (primarius ENT/genital) and primary squamous cell carcinoma of the lung. The context of clinical and radiological findings may support correct classification, but sometimes this is not successful even with the help of such information. Therefore, we are currently developing a whole transcriptome sequencing (WTS) based classifier for the diagnostic classification of lung lesions (nodules), which, in addition to the correct entity assignment by gene expression profile, also provides clinically relevant biological information, such as the activation state of relevant signal transduction pathways, the detection of viral genome and the immunological landscape of the tumor. Over 200 cases of NSCLC, pulmonary metastasis of other primary cancer types, and samples of corresponding non-pulmonary primary tumors have been analyzed so far and incorporated in the classifier. Preliminary data show strong differentiation capability between the different tumor types. The compilation and size of the sample set is still ongoing to further enhance robustness and performance.

Abstract No. 724

Upregulation of Serpin B13 in squamous cell carcinomas of the lung predicts favorable prognosis

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Objective: Therapy of NSCLC has made immense progress in recent years. However, there are much more options for pulmonary adenocarcinomas than for squamous cell carcinomas. For the latter, valid prognostic markers in addition to common markers such as TNM and grading are also lacking. Serpin B13 (hurpin, headpin) belongs to the superfamily of serpins and acts as a serine proteinase inhibitor inhibiting cathepsin-K and -L suggesting a possible role in metastasis. Serpin B13 is a cytoplasmic protein that plays a role in the proliferation and differentiation e.g. of keratinocytes. Here, we describe serpin B13 expression at RNA- and protein level as well as epigenetic modifications and examine the impact on overall survival using lung squamous cell carcinoma tissues. **Methods:** Illunina bead chips were used to analyze epigenetic modifications of the serpin B13 gene in NSCLC; expression on RNA-level was assessed by transcriptome analysis using an Agilent platform. Expression of serpin B13 on the protein level was assessed by immunohistochemistry using an independent cohort of 125 patients and correlated with overall survival and immune infiltration. **Results:** We revealed a significant difference in methylation of serpin B13 loci in NSCLC and showed that mRNA- and protein expression of serpin B13 is upregulated in squamous cell carcinomas. High protein expression of serpin B13 was significantly associated with a better overall survival. Moreover, carcinomas with low immune infiltration showed significantly less protein expression than carcinomas with high immune infiltration. **Conclusions:** Our results suggest serpin B13 to be a novel prognostic biomarker for lung squamous cell carcinomas. Its implementation in future routine diagnostic workup appears reasonable.

Abstract No. 725

An interpretable deep learning autoencoder unravels that coagulation-associated genes are significant differentiators between adenocarcinoma and small cell lung cancer

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Adenocarcinoma and small cell lung cancer are two major distinct subtypes of lung cancer, together accounting for 50%-65% of all lung cancer cases. Although these two lung cancer subtypes have different risk factors, histological features, and treatment approaches, which often facilitate tailored medical decisions, a comparison of their transcriptomic profiling can still benefit research in multiple ways. These benefits may include elucidating molecular mechanisms and potential therapeutic targets, discovering novel biomarkers for clinical application, or identifying key gene expression patterns to explain their distinct pathological characteristics.

Although the traditional methods of differential expression and gene set enrichment analysis have been used to compare cancer samples, there is great potential at the moment for more sophisticated deep learning (AI) based methods to bring a novel perspective to these types of questions. In fact, we have developed a novel adaption of the variational autoencoder architecture which greatly increases the biological interpretability of transcriptomes. This deep learning model was trained on 27,887 transcriptomes from various biological sources.

An in-depth exploration of the lung cancer subtypes using this model shows that among the major biological processes defined in the set of so-called Hallmark Pathways, *coagulation* was found to be a top differentiator between adenocarcinoma and small cell lung cancer. This finding is supported by relevant publications in the field. Although further laboratory validation will be necessary to enhance the credibility of this conclusion, this preliminary result already offers valuable insight into future research directions and has the potential to be generalized to a wider range of studies.

Abstract No. 726

Human Lung Multi-omic Cell Atlas, a resource to advance translational lung cancer research

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Cancer initiation and progression result from the accumulation of genetic and epigenetic alterations. Altered epigenetic landscapes provide valuable insights into the molecular characterization of non-small cell lung cancer (NSCLC), as they contain tumor and cell-of-origin-specific signatures. Lung tumors are multifocal and supported by a reprogrammed stroma that influences therapeutic response. Personalized therapies lead to the development of resistance, often of unknown mechanisms. Furthermore, >50% of patients do not harbor genomic aberrations with a clear therapeutic strategy. These gaps highlight the urgent need for a better molecular understanding of NSCLC. Therefore, we set out to build a human lung cell multi-ome atlas by providing a data resource of lung cells from healthy lung, NSCLC and tumor-adjacent normal tissue. We initiated -omics profiling with multi-region single-cell RNA-seq and array-based DNA methylation of seven adenocarcinoma patients. We identified 4 tumor cell clusters in 6 out of 7 donors. Differential expression analysis revealed 23 epigenetic regulators altered that classified the 4 tumor clusters. In addition, 144 epigenetic regulators involving 13 different cell types were deregulated in the tumor regions. Interestingly, some were altered in multiple cell types, suggesting a possible pan-cellular epigenetic regulation in the tumor microenvironment (TME). Taken together, the observed alterations may promote epigenetic heterogeneity in tumors and TME. Next, the atlas will be complemented with proteomic profiling and spatial epigenetic analyses of tumor and TME. This comprehensive multi-omic resource is expected to serve as a solid foundation for advancing translational lung cancer research.

Contribution of a Disease-spanning Working Group: Single Cell Analysis

Abstract No. 727

Integrated analysis of DNA methylation and transcriptome revealed distinct metabolism patterns of tumor-associated macrophages in lung cancer

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Despite the comprehensive DNA methylation studies in lung cancer cells, the impact of DNA methylation in the lung tumor microenvironment (TME), especially in tumor-associated macrophage (TAMs) as the most abundant immune cells in lung TME remains imprecise. This study is designed to decipher the role of DNA methylation in TAMs differentiation and activation during lung tumor establishment and progression**.** Towards this aim, TAMs were sorted and isolated with multispectral flow cytometry from lung tumors and healthy tissues. DNA methylation patterns were mapped through the Infinium HumanMethylation850 BeadChip from Illumina. Interestingly, TAMs showed a distinct DNA hypermethylation pattern compared to normal macrophages. To identify the potential relevance between DNA methylation changes and cellular functions, BioCyc pathway analysis showed the promoters of differentially methylated regions (DMRs) were mainly associated with fatty acid (FA) metabolism signaling. Integrative analysis of DNA methylation and transcriptomic profile of TAMs indicated FA metabolism-associated genes were significantly downregulated alongside with up-regulation of the glycolysis pathway. Along the same line, the transcription factors (TF) binding analysis revealed that DNA hypermethylation mainly occured in the binding sites of FA metabolism-associated TFs. Finally, our *in vitro* TAMs system demonstrated the hypermethylation of DNA rendered the pro-tumor phenotypes to TAMs during crosstalk with lung cancer cells. Altogether, for the first time, we showed that TAMs have a distinct DNA hypermethylation profile which can regulate specifically the FA metabolism genes expression machinery. The findings could help develop new therapeutic strategies targeting the metabolism of TAMs in lung cancer.

Abstract No. 728

Targeting Immunotherapy Resistance in Small Cell Lung Cancer: Insights from CRISPR-Induced Preclinical Mouse Models

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Small cell lung cancer (SCLC) is an aggressive neuroendocrine (NE) cancer defined by universal loss of the tumor suppressors *TP53* and *RB1* and a high degree of neuroendocrine heterogeneity, with NE^{high} and NE^{low} subtypes displaying distinct therapeutic vulnerabilities. To uncover the mechanisms of SCLC development and therapy responses, preclinical mouse models that resemble the genetic features of the human disease are essential. We have developed a flexible toolkit to assemble recombinant CRISPR adenoviruses (AV) that target the murine homologs of the human SCLC driver genes. By infecting immunocompetent mice intratracheally with these CRISPR AVs, we have induced the efficient development of genetically defined NE^{high} SCLC tumors that are suitable for preclinical immunotherapy studies.
Although SCLC tumors feature a high tumor mutational burden, SCLC patients rarely show a durable response to immune checkpoint inhibitors (ICI). Retrospective patient studies have shown that the few ICI responses are mostly limited to NE^{low} SCLC subtypes, while the vast majority of SCLCs are NE^{high} and refractory to ICI due to defects in loading major histocompatibility complex class I (MHCI) molecules with neoantigens. Here, we report the identification of a small molecule that activates Notch signaling in NE^{high} SCLC cells, inducing NE^{low} lineage-specifying transcription factors and switching them to a NE^{low} phenotype. Most importantly, this treatment is associated with massive induction of MHCI expression, suggesting a restoration of the antigen presentation machinery. These results warrant further preclinical studies of this compound as an SCLC treatment for boosting immunotherapy responses, potentially leading to a durable ICI benefit also for NE^{high} SCLC patients in the clinic.

Abstract No. 729

Association of IL-22 and IL-17 in advanced non-small cell lung cancer (NSCLC) with no metastasis, oligo-metastasis and diffuse metastasis

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Background: Despite advance in treatment strategies, the current prognosis of patients with advanced non-small cell lung cancer (NSCLC) is still very negative. However, due to immunotherapies, targeted therapies and local therapies the 5-year survival of an advanced state of lung cancer with oligo metastasis increased in some studies from 10% to 15-30%. Unfortunately, these options do not work for all patients, and resistances can develop during treatment. Reasons for this are still not fully understood, however pathways in the microenvironment of the carcinoma could be one reason.
Methods: We retrospectively collected tissue samples of NSCLC patients with advanced adenocarcinoma who were treated at the LMU Klinikum in Munich, Germany. We stained interleukin-17 and interleukin-22 in these samples. Samples were also stained with hematoxylin and eosin to identify tumor tissue. Patients got divided into three groups: patients with no-metastasis, oligo-metastasis, and diffuse metastasis.
Results: Primary lung tissue of 173 people with adenocarcinoma was examined. No association between IL-17 and metastasis category was found, however there was a significant higher amount of IL-22 in tissues of lung cancer with diffuse metastasis compared to tissues without metastasis.
Discussion: IL-22 is of interest as a prognostically relevant biomarker or as a potential therapeutic target. Antibodies against IL-22 or part of its pathway are currently being investigated.

Abstract No. 730

Spatial signatures in the EGFR/KRas mutation-driven lung cancer

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Immune checkpoints therapies have become revolutionized in various cancers including lung cancer. However, only 20% of lung cancer patients respond to immunotherapies. The heterogeneity of the lung TME is influenced by not only tumor type (NSCLC or SCLC) but also mutation-driven cancer. It is therefore decisive to understand TME composition, the spatial architecture to gain new insights. Here we used high plex mIF studies to evaluate the spatial architecture in EGFR/KRas mutation-driven lung tumors. We established a lung adenocarcinoma patient-derived cohort from 200 patients. Tumor microarrays (TMAs) prepared from these tumors were subjected to high plex mIF with 42 antibodies (structural, immune, functional) and imaged using Phenocycler™-Fusion. We further established computational workflow after HALO-image analysis for cell segmentation and annotated 40 phenotypes to calculate distribution, as well as spatial proximity in tumors. Spatial distribution analysis showed altered immune signatures (eg: PD-L1, TIGIT, HLA-DR, CD8, Pax5, and PD1), high proliferation, and less apoptosis in EGFR and Kras mutated tumors. In addition, t-SNE and UMAP analysis of 2 million cells showed 36 EGFR/KRas mutation-specific clusters in tumors. Notably, neighborhood analysis showed 20 different clusters in EGFR/KRas mutation with specific phenotype. Proximity analysis showed that high Tregs and low CD8-CTLs in the tumor cell neighborhood. In addition, overall and disease-free survival analysis showed that PD-L1, HLA-DR, CD68, and TIGIT are positively correlated with hazard. This study shows that ultra-high plex mIF on tumors combined with adequate computational tools provide an opportunity to understand the spatial architecture of lung cancer, and to improve therapeutic targets.

Abstract No. 731

SMAD2 splice variants - driver or consequence of EGFR-TKI resistance in NSCLC?

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Aberrant TGFβ signaling is associated with progression of NSCLC. Within the TGFβ pathway, signal transduction occurs via C-terminal receptor-mediated phosphorylation of SMAD2 and SMAD3. In addition, SMADs can optionally be phosphorylated within the linker domain by intracellular kinases such as ERK1/2, allowing integration of non-TGFβ signaling pathways.
Phospho-SMAD2 linker (pSMAD2L) occurs in a mitosis-dependent manner in NSCLC cell lines, and NSCLC patients with low pSMAD2L^{+} cell density showed poorer overall survival. A549 cells constitutively lacking pSMAD2L (A549L^{sub}) exhibited reduced migration, proliferation and altered TGFβ1-dependent gene expression. In addition, frequency of alternatively spliced short SMAD2 (SMAD2$^{\Delta E3}$) was increased compared to wild-type cells. SMAD2$^{\Delta E3}$ is essential for embryogenesis in mice, whereas full-length SMAD2 (SMAD2^{FL}) is negligible. However, pSMAD2L was significantly decreased in H1975 cells carrying *EGFR* double mutation (*L858R/T790M*) and exhibited increased SMAD2$^{\Delta E3}$ levels compared to other NSCLC cell lines. In contrast, H1650 cells with *EGFR exon19 deletion* expressed comparable pSMAD2L and SMAD2$^{\Delta E3}$ abundances to NSCLC cell lines with wild-type *EGFR*. Upon stimulation with the EGFR-TKI Gefitinib, pSMAD2L abundance decreased and SMAD2$^{\Delta E3}$ increased in sensitive H1650 cells, whereas H1975 Gefitinib-resistant cells responded in the opposite manner.
We will next investigate whether de-phosphorylation of pSMAD2L occurs via specific phosphatases and the role of SMAD2$^{\Delta E3}$ in NSCLC. Therefore, we will generate NSCLC cell lines exclusively expressing SMAD2^{FL} or SMAD2$^{\Delta E3}$ in different *EGFR* mutation backgrounds to explore cellular consequences and further elucidate the specific roles of both SMAD2 splice variants in acquired EGFR-TKI resistance.

Abstract No. 732

Survival differences by gender for resected small cell lung cancer

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Introduction:
SCLC accounts for about 15% of all lung cancer cases. About 30% have localized tumors,only 5% of them are eligible for surgical treatment. The survival rate with SCLC is very low. Surgery may play a role in selected patients.
Studies have shown that survival of women with SCLC is more favorable. However,the prognostic significance of gender has not been thoroughly investigated. Therefore,we analyzed if gender affects survival after resection of SCLC.

Method:
This retrospective single center analysis between 07/2000-10/2021 included clinical data of 163 patients with SCLC treated by resection within a multimodal concept. The surgical procedure, perioperative management,perioperative outcomes,overall survival(OAS) and survival by tumor stage were identified and analyzed.

Results:
Of the 163 patients,92(56.4%) had "very limited",65(39.9%) "limited" and 6(3.7%) "extensive" disease. The distribution of tumor stage,as well as ECOG score,were almost equal. Mean age at the time of surgery was 62.6±10.3(female) and 62.6±3.1(male)years. 54(33.8%) of patients were female.
There was no significant difference regarding the resection (anatomical resection in 82%). Median follow-up was 33.4m with a follow-up rate of 93.3%. The 90d survival rate was 96.3%(female) and 93.4%(male), respectively. OAS (59.1vs.21.7m) and stage-specific survival was also significantly higher in women.

Conclusion:
Our results show that there are significant gender differences in survival in patients with SCLC in favor of female. Both overall survival and stage-specific survival of surgically treated female SCLC patients was significantly better. We plan to conduct further and detailed studies to elucidate the causes of the observed differences.

Abstract No. 733

Integration of ATACseq and RNAseq identifies key regulators in lung tumor-associated macrophages

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Tumor associated macrophages (TAMs) are highly dependent upon the tumor microenvironment. TAM subgroups display phenotypic and functional specialization in gene and TF expression. To assess the dynamic regulation of epigenetic and transcriptional programs in TAMs, we FACS sorted primary human macrophages from lung tumor (TAMs) and non-tumor (NMs) and performed "Assay for Transposase-Accessible Chromatin with high throughput sequencing" (ATACseq) and mRNAseq, followed by integrative analysis. An integrative study found 376 open and 219 closed chromatin areas that promote or reduce gene expression in TAMs compared to NMs, respectively. Pathway study of genes surrounding TAMs' more accessible chromatin showed involvement in ECM-receptor interaction, PI3K-AKT, PDGF, Notch, p53, HIF1A signaling, angiogenesis, integrin signaling, developmental biology, etc. We also utilized TOBIAS, a comprehensive, accurate, and quick footprinting platform, to discover transcription factors (TFs) that may recruit chromatin remodeling enzymes to alter TAMs chromatin landscape. 28 TFs (SP2, SP1, THAP1, CTCFL, SP3, E2F4, KLF3, PATZ1, SP4, NRF1, etc.) increased binding in accessible chromatin of TAMs, whereas 20 TFs (PPARA, DBP, CEBPA, CEBPG, ATF4, RXRG, PPARG, CEBPB, NKX6-1, HLF, etc.) decreased binding. Macrophage Enhancer Atlas 2.0 showed 15 of 48 TFs with substantial binding changes operate as enhancers and super-enhancers in TAMs. The functional assessment of TAMs-specific altered chromatin regions and TFs will further provide opportunities to understand and manipulate the epigenetic status of human lung TAMs, contributing to human medicine in terms of diagnostic and therapeutic modalities.

Contribution of a Disease-spanning Working Group: Lung Environment Interaction

Abstract No. 734

3D co-culture model of bronchial epithelial cells with immune cells

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The airway epithelium is a pseudostratified layer of mainly basal, secretory, and ciliated cells. Basal cells are progenitor cells for the other epithelial cells and are found in humans down to the respiratory bronchi, while in mice they are exclusively located to the trachea. This differentiation process can be recapitulated *in vitro* by culturing basal cells under realistic conditions at the air-liquid interface (ALI) or in matrigel as organoids. In multiple diseases, including lung cancer, fibrosis, or COPD, the immune system plays a crucial role in interacting directly or indirectly with epithelial cells, influencing disease development. For this reason, we aim to develop a 3D cell culture model in which epithelial cells are co-cultured with immune cells. We established primary mouse tracheal epithelial cell (mTEC) culture and differentiation. mTEC cells isolated from FVB (WT, tomato or KRT5-lineage traced) mice were KRT5+ during basal cell expansion. After differentiation for 28 days at ALI, acTub+ ciliated cells, CC10+ Club cells, and MUC5B+ goblet cells were identified by IF, proving efficient differentiation. In matrigel, mTEC cells showed well formed bonchospheres with clear visible lumen and cell polarization. The immune cells (monocytes, NK cells or CD8+ cells) were isolated from FVB mice and either applied apically or basolateral in ALI to mimic direct and indirect cell interaction, respectively. Similarly, we are planning to co-culture the cells in matrigel. With these techniques, we will be able to analyze the influence of immune cells on epithelial cells and vice-versa in healthy and disease simulated conditions. This will lead to a better understanding of disease development and progression and will give insight into new target options for treatment.

Contribution of a Disease-spanning Working Group: Lung Environment Interaction

Abstract No. 735

Reactivation of relevant tumor suppressors in cancer cell lines via dCas9 mediated Epigenetic Editing

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Epigenetic modifications play a crucial role in regulating gene expression, and aberrant epigenetic changes can contribute to the development of cancer, such as DNA hypermethylation of tumor suppressor CpG islands. Recently, epigenetic editing using nuclease-inactivated dCas9 fused with effector domains such as TET1 (or DNMT) has emerged as a powerful tool to modify specific epigenetic DNA marks and potentially reactivate candidate tumor suppressors.
In this study, we investigated the effectiveness of using epigenetic editing to target specific tumor suppressor genes in human cancer cell lines. We designed guide RNAs to target the promoters of several candidate tumor suppressors and used nuclease-inactivated dCas9 fused with TET1 (or other effectors such as DNMT3A, VP160, p300) to modify tumor suppressor expression. We found that targeted epigenetic editing resulted in reactivation of silenced tumor suppressor genes and reduced cell proliferation in several human cancer cell lines.
Our results demonstrate that epigenetic editing using nuclease-inactivated dCas9 and epigenetic effectors can be a highly effective and a targeted approach to reactivate silenced tumor suppressor genes. This technique has the potential to be a valuable tool in cancer research and therapy by restoring the normal function of epigenetically silenced genes.

Abstract No. 736

Spatial single-cell metabolic and epigenetic profiling of NSCLC

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The lung is a complex organ composed of over 50 different cell types. This complexity is also reflected in non-small cell lung cancer (NSCLC). NSCLC is the leading cancer-related death worldwide, and one of the biggest challenges scientists and clinicians face is the high inter- and intratumor heterogeneity. Tumor heterogeneity complicates diagnosis and treatment, indicating the need for a greater understanding of NSCLC. To get new insights we use Multiplexed Ion Beam Imaging (MIBI), a novel imaging technology that enables high-dimensional single-cell spatial proteomics analyses of formalin-fixed paraffin-embedded tissue. More than 40 metal-conjugated antibodies are multiplexed to image cell-specific protein expression with subcellular resolution. It allows the quantification of proteins and posttranslational modifications with a dynamic range that spans five magnitudes. Furthermore, it enables cell classification and detection of cellular niches and interactions in tumors, adjacent tissue and distal lung. This multiregional profiling will capture heterogeneity in a spatial context, the diverse tissue structures and multicellular communities. Using antibodies against metabolic regulators, epigenetic players and epigenetics modifications allows the investigation of the interplay between cellular metabolism and epigenetics. This will give insights into how these pathways influence each other at the single-cell level and how these processes are regulated on a spatial level. These results will enable us to identify signatures promoting tumorigenesis in the tumor and surrounding stroma. Altogether, the collected data will contribute to the molecular characterization of NSCLC and advance translational lung cancer research.

Contribution of a Disease-spanning Working Group: Single Cell Analysis

Abstract No. 737

Expression of markers of immune exhaustion is shared between multiple cell types in early-stage NSCLC

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Background: Therapy resistance and tumor immune evasion, for example as a result of immune cell exhaustion, are limiting the success of individualized therapies in NSCLC patients and are associated with poor prognosis. Correlations between different markers of immune dysfunction and exhaustion and their specificity for certain cell types is not yet fully understood.

Methods: We selected eight immune-associated molecular exhaustion markers in tumor and draining lymph nodes. We carried out correlation analyses (molecular, histological, blood-based and clinical variables) to identify potential relationships.

Results: Among the clinical parameters investigated, only performance score and pre-existing oncological diagnoses were associated with increased levels of immune exhaustion markers such as CD276 (p=0.008) and IDO1 (p=0.012). TIM-3 and CD276 expression was correlated in N1 (p=0.001) and N2 lymph nodes (p=0.002). These two markers each showed a correlation to expression markers of three different cell types: TIM-3 to CD19 (B-cells), CD14 (monocytes) and CD4 (T helper cells); and CD276 to CD19 (B-cells), CD14 (monocytes) and CD8 (cytotoxic T-cells).

Conclusion: Our data confirm the relevance of immunological exhaustion in NSCLC tumor and efferent lymphatic tissues. Immune cell exhaustion of B-cells and monocytes will be investigated more in-depth in further analyses.

Abstract No. 738

JNK is activated in an EGFR-induced Drosophila lung tumor model and promotes cancer progression

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Lung cancer is the leading cause of cancer-related mortality. Mutations in the EGFR gene are among the most important drivers of lung tumor development, but the success of personalized therapies is still limited due to the development of resistance. JNK plays an important role in the development of lung cancer. The role of JNK in lung cancers harboring EGFR gene mutations is not fully understood. We specifically expressed constitutively active EGFR ($EGFR^{CA}$) in the *Drosophila* airway system and performed a comprehensive phenotypic analysis. Ectopic expression of $EGFR^{CA}$ induced tumor-like growth of larval tracheal stem cells and thickening of the tracheal epithelium, leading to early death. We found that overexpression of EGFR in larval trachea activated three canonical downstream signaling pathways: MAPK, PI3K/Akt, and JAK/STAT signaling pathways, respectively. We blocked each of the three signaling pathways and found that in this EGFR ectopic expression-induced lung cancer model, the MAPK pathway was mainly involved in proliferation, PI3K/Akt in maintaining cell survival, and JAK/STAT in metastasis and invasion. We next found that JNK is activated in the EGFR-induced tumor model and blocking JNK rescued tumor-like proliferation and significantly reduced EGFR activation of MAPK, PI3K, and JAK/STAT pathways, while also rescuing EGFR-induced animal death. Taken together, we developed a Drosophila lung cancer model with tumor-like growth of tracheal stem cells and used the Drosophila model study to identify JNK as a key regulator of EGFR-induced lung cancer progression, providing a promising therapeutic option for treating lung cancer patients with EGFR gene mutations and JNK overexpression.

Abstract No. 739

Donor-dependent immune responses in interleukin-2-stimulated human lung slices

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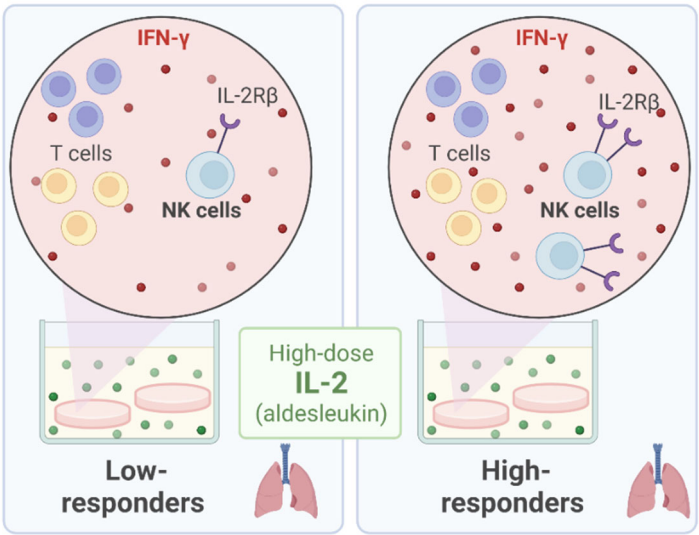
The use of high-dose interleukin-2 (hdIL-2) treatment as an anti-cancer therapy has suffered drawbacks due to patient-specific severe side effects, including lung toxicity. Still, pathophysiological mechanisms of IL-2-induced lung toxicity need clarification to ensure safety of future IL-2-based or other therapies.

To identify possible key cells and molecules involved in IL-2-induced lung toxicity, human precision-cut lung slices (PCLS) were stimulated with hdIL-2 (aldesleukin) for 5 days. Immune responses were investigated using flow cytometry and ELISA. Lactate dehydrogenase (LDH) was measured to determine toxicity.

HdIL-2 stimulation of PCLS induced secretion of IFN-γ and LDH. IFN-γ secretion was saturated at clinically relevant IL-2 doses in low-responders but was further increasing up to 30-fold at higher doses in high-responders, indicating overshooting immune responses. While hdIL-2 stimulation of PCLS did not change prevalence of T cells, NK cell frequencies were especially increased in high-responders (up to 4-fold increase for high-responders vs. 1.5-fold increase for low-responders). NK cells of high-responders showed higher IL-2 receptor β (IL-2Rβ) expression under control conditions.

Our data show donor-dependent immune responses of lung-resident cells towards hdIL-2 which might be accounted for by differences in NK cell populations. Future studies will be needed to investigate donor-dependent NK cell activation in the context of lung toxicity during IL-2 therapy.



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Abstract No. 740

Developmental origins of cell heterogeneity in the human lung

Alexandros Sountoulidis

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The lung contains numerous specialized cell-types with distinct roles in tissue function and integrity. To clarify the origins and mechanisms generating cell heterogeneity, we created a first comprehensive topographic atlas of early human lung development. We report 83 cell states, several spatially-resolved developmental trajectories and predict cell interactions within defined tissue niches. We integrated scRNA-Seq and spatial transcriptomics into a web-based, open platform for interactive exploration. To illustrate the utility of our approach we show distinct secretory and neuroendocrine states, largely overlapping with the programs activated during lung fibrosis or small cell lung cancer progression[JT1] . We define the origin of airway fibroblasts associated with airway smooth muscle in bronchovascular bundles and describe a trajectory of Schwann cell progenitors to intrinsic parasympathetic neurons controlling bronchoconstriction. Our atlas provides a rich resource for further research and a reference for defining deviations from homeostatic and repair mechanisms leading to pulmonary diseases.

Contribution of a Disease-spanning Working Group: Single Cell Analysis

Abstract No. 741

Transcriptomic signatures of malignant pleural effusions as hints to diagnosis and prognosis

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Pleural effusions (PE) are associated with a great variety of diseases and affect ~2 million people annually worldwide. Though crucial for patient treatment, the identification of the underlying cause is cumbersome and time consuming. We hypothesize that human pleural effusions reveal cellular and molecular fingerprints, which could serve as hints for the distinction of PE diagnosis and prognosis. Therefore, clinical data and pleural effusions from patients with benign or malignant PE were collected and a total of 22 samples were subjected to bulk RNA sequencing [n=3 benign PE (BPE), n=4 malignant pleural mesothelioma (MPM), n=8 lung adenocarcinoma (LUAD), and n= 7 various metastatic cancers (MCA)]. A novel algorithm-based mutational analysis method, developed by our group, revealed an increased number of mutations in the malignant samples and a great variation between these patients. Overall, patients with MPM presented the lowest number of mutations (SNVs, insertions and deletions) and MCA patients the highest. Also, clustering and hypergeometric distribution analysis revealed the MPM group to be 1,73 fold over-represented (P=0,085) in one of the clusters. In all malignant groups transition mutations were clearly overrepresented, and all T to C trinucleotide changes displayed a significantly different contribution in the MPM group when compared with the other malignant groups. Apart from expanding our cohort we are currently performing in depth gene expression and pathway analysis that will allow us to discover diagnostic and prognostic signatures of PE in the future.

Abstract No. 742

Predictors of overall and progression-free survival in oligometastatic non-small cell lung cancer

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Background:
Oligometastatic non-small cell lung carcinoma (OMD-NSCLC) as a newly categorized entity presents a clinical challenge in regards to definition, potential therapy strategies and prognostic factors associated with survival. Therefore in this study we aimed to analyze patients with OMD-NSCLC and identify factors associated with overall survival (OS).

Methods:
Our retrospective study included all patients with OMD-NSCLC treated at LMU University Hospital from 2015 to 2020. OMD was defined as having between one and five metastases in up to three organ systems at first diagnosis of NSCLC. Serosal metastases were excluded. We used univariate Cox regression to identify factors possibly associated with OS and PFS ($p<0.1$), which were subsequently included in multivariate Cox regression analysis.

Results:
The overall median survival time (mOS) was 24.2 months [95% CI: 15.4 to 33.0]. In multivariate analysis we found increasing age (HR: 1.05, $p=0.004$) to be associated with worse OS. T3 vs. T4 (HR: 0.16, $p=0.001$), T2 vs. T4 (HR: 0.10, $p<0.0001$), and T1 vs. T4 (HR: 0.19, $p=0.001$) was associated with better OS. Additionally, radiotherapy of the primary tumor was associated with better OS (HR:0.32, $p=0.004$), whereas surgical resection of the primary tumor did not reach significance (HR:0.37, $p=0.14$). First line platinum plus immune therapy was associated with prolonged OS (HR:0.23, $p=0.02$).

Conclusion:
In patients with OMD-NSCLC local tumor control especially via radiotherapy seems to be an important factor for prolonged OS and PFS. Systemic first-line therapy with platinum plus immune therapy can further prolong survival. Biomarker analyses to better understand metastasis and relapse, as well as multicenter analyses in BZKF, are ongoing.

Abstract No. 743

miR-193b promotes lung cancer progression by interfering with macrophage activation

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Cancer cells regulate several miRNAs in immune cells to limit their antitumor response and reprogram them to promote tumorigenesis. The molecular cues that control the interaction of immune and tumor cells in the lung tumor microenvironment are only partially understood. Using a miRNA array-based approach we found that miR-193b is highly induced in macrophages co-cultured with tumor cells. Further, we could confirm the upregulation of miR-193b in tumor-promoting M2-like macrophages. Additionally, we could show that after macrophage depletion using the MaFIA-mouse model a significantly decreased level of miR-193b indicating a macrophage-dependent expression. Further, inhibition of miR-193b by a hairpin inhibitor drives macrophage activation towards the pro-inflammatory phenotype. Conditioned media from miR-193b inhibitor-treated macrophages significantly reduced tumor cell proliferation and migration, while inhibition of miR-193b in tumor cells alone does not appear to have an impact on tumor cell behavior. By using a transgenic mouse model, we were able to knock out miR-193b in monocytic cells. Notably, we observed a decreased tumor burden in tumor cell injected (miR-193b$^{fl/fl}$LysM^{Cre}) and oncogene-driven (KRas^{LA2}miR-193b$^{fl/fl}$LysMcre^{Cre}) myeloid cell-specific miR-193b tumor-bearing mice. Additionally, we found an altered immune cell composition as well as reduced tumor cell proliferation and vessel formation in this myeloid cell-specific miR-193b mice model. In conclusion, our findings have important implications for the development of targeted immunotherapies for lung cancer. Targeting miR-193b in macrophages and related pathways could potentially reverse the tumor-promoting effects of these cells in the tumor microenvironment and enhance the anti-tumor immune response.

Abstract No. 744

Therapy with immune checkpoint inhibitors: a propensity score matched analysis of expenditures, hospitalizations and survival from claims data

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Therapy with immune checkpoint inhibitors (ICI) for lung cancer was introduced in Germany in 2015 in second line, leading to significantly improved survival outcomes. In this analysis we aimed to assess the effect of ICI therapy on survival expenditures and hospitalizations.
We analyzed claims of 15.923 lung cancer patients with metastases diagnosed in 2015 and 2016. We used 1:1 propensity score matching of age, year of incidence, metastases status (M1a, M1b, or M1c), population density at residence, and hospital size to match patients with and without ICI therapy. We compared expenditures (three years), hospitalizations, number of hospital nights, and survival between patients with and without ICI using means with standard deviation and paired t-test for numeric variables, absolute and relative frequencies with McNemar-test for categorical variables, and Kaplan-Meier curves with Log Rank-test to compare survival.
We found matches for 330 patients (1.6%) with ICI in our dataset. Kaplan Meier curves showed significantly improved survival for patients with ICI therapy compared to patients without ICI therapy (median survival: 26 months vs. 10 months, $p < 0.0001$). Overall expenditures for patients with ICI therapy were significantly higher compared to patients with other therapies (129,451€ vs. 58,740€, $p<0.0001$). When adjusted for survival time expenditures per month did not differ significantly anymore (5,250€ vs. 5,619, $p=0.11$). The number of outpatient hospitalizations (5.3 vs. 1.2, $p<0.0001$), hospital stays (13.4 vs. 7.2, $p<0.0001$), and hospital nights (172.0 vs. 62.9, $p<0.0001$) was significantly higher in ICI therapy patients.
Patients treated with second-line ICI monotherapy generate higher hospital utilization and expenditures but also longer survival.

Abstract No. 745

Metabolic vulnerability of TKI-resistant EGFR-mutant lung adenocarcinoma

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In our previous work, we demonstrated the potential of exploiting a causal link between aberrant mTOR activity and chemoresistance in NSCLC as a new therapeutic strategy for CDDP-refractory tumor cells. Specifically, we found that mTOR mediated suppression of autophagy, a self-digestion program that helps cells cope with energy stress induced by metabolic perturbation, could sensitize tumor cells to metabolic drugs. Building on these findings, we sought to investigate the role of autophagy defects in TKI-resistant EGFR-mutated lung adenocarcinomas, using PC-9 cells as a model.
Consistent with the idea that impaired autophagy contributes to metabolic sensitivity, we found that ATG knockout PC-9 cells displayed metabolic vulnerability. Additionally, immunohistochemical analysis of relapsed EGFR-mutant NSCLC patients showed markers of autophagy impairment.
To mechanistically investigate the potential link between TKI resistance and metabolic vulnerability, we used three different approaches to make PC-9 cells resistant to TKIs. First, using CRISPR/Cas9, we introduced typical EGFR-dependent mutations (T790M, C797S, and T790M/C797S), which confer resistance to erlotinib and osimertinib. Second, we lentivirally transduced genes that induce bypass signaling or activate downstream pathways to confer TKI resistance. Finally, we generated PC-9 cell clones resistant to all generations of TKI inhibitors through dose escalation.
In general, with all of these approaches, we observed a strong correlation between TKI resistance and metabolic sensitivity.
Our data suggest that acquired resistance to a variety of anticancer drugs induces a metabolically vulnerable state that is mechanistically explained by autophagy defects and that can be exploited to treat resistant cancer patients.

Abstract No. 746

I3LUNG: Integrative Science, Intelligent Data Platform for Individualized LUNG Cancer Care With Immunotherapy

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Only 30-50 % of patients with advanced non-small cell lung cancer (aNSCLC) and treated with Immunotherapy (IO) achieve long-term benefit. To date, Programmed Death-Ligand 1 (PD-L1) is still the only biomarker to select patients for IO, with limited success. Therefore, it is of great relevance to get more insight to understand the complexity of the immune system tumour microenvironment, not only in tumour-tissue of the patients, but also in the blood and microbiome. Artificial Intelligence (AI) and machine learning (ML) frameworks utilize to develop a multi-national platform to predict the patient´s response to IO. In the retrospective part, a platform is build combining clinical data, collected from 2.000 patients who have received or will receive IO. Clinical data, molecular characteristics, together with treatment patterns are put together to generate a predictive model for IO. This model will be used to validate the AI models and generates a multi-omics prospective data collection for 200 aNSCLC patients in the prospective part to identify biomarkers predicting response to IO.
Moreover, a psychological sub-study analysing the impact of AI in patients-physicians interactions and common engagement in treatment decisions to improve co-decisions by the patients complete the study.
The aim of I3LUNG is to develop an integrated AI-based predictive tool to support the best possible personalized therapy, improve survival, reduce excessive toxicity, maintain quality of life (QoL) and emotional health of patients with aNSCLC.
Finally, the constructed novel AI-assisted data storage and elaboration platform, guiding IO treatments, could help to optimize timing and cost-effectiveness of IO-treatment decisions [1].

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Abstract No. 747

Receptor turnover and feedback control are key mechanisms shaping the cell type-specific dynamics of TGFβ signaling in lung cancer

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Non-small-cell lung cancer (NSCLC) is the leading cause of cancer-related death globally, and elevated levels of the Transforming Growth Factor-beta (TGFβ) ligand have been linked to poor patients' survival. However, the underlying mechanisms that contribute to the pro-tumorigenic effects of TGFβ in NSCLC are not fully understood.
To better understand the properties that shape TGFβ-induced signal transduction in lung cancer cells, we studied three NSCLC cell lines (H1650, H838, and H1975) that carry mutations commonly observed in lung cancer patients, along with an epithelial cell line (Beas-2B) as a control. To extent our dynamic pathway model of TGFβ-induced signal transduction that we calibrated based on through time- and dose-resolved immunoblotting data, inhibitor treatment and target gene expression, we established a targeted mass spectrometry approach that allows us to perform absolute quantification of the core components of TGFβ-induced signal transduction including both receptor subtypes. Surprisingly, whereas the amounts of SMAD proteins are comparable in the NSCLC, the receptor levels show major alterations. Furthermore, the key negative regulator SMAD7 although strongly induced at the mRNA level in response to TGFβ is barely detectable at the protein level. These quantifications were extended to tumor-free, tumor-rim and tumor samples from a cohort of NSCLC samples which confirmed that both receptor types' abundance differs significantly in the NSCLC cell lines and showed that tumor rim samples are highly heterogenous and resemble in parts more the tumor or tumor-free samples. These results highlighting that the TGFβ receptor is one of the most sensitive nodes controlling pathway activation and could inform future therapeutic strategies for NSCLC.

Contribution of a Disease-spanning Working Group: Lung Environment Interaction

Abstract No. 748

Characterization of FGFR1-4 variants-of-unknown-significance in the framework of the nNGM Preclinical Platform

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Background: Lung Cancer is the leading cause of death among cancer patients in Germany. Mutations in any of the four Fibroblast Growth Factor Receptors (FGFR1, FGFR2, FGFR3, FGFR4) individually occur with estimated frequencies of 1 – 2% in non-small cell lung cancer (NSCLC) patients. Mutations outside of mutational hotspots or the tyrosine kinase domain are commonly classified as variants-of-unknown-significance (VUS) due to insufficient information about their functional impact. Preclinical testing of such mutations in suitable model systems can provide functional information about their oncogenic potential and possible treatment options.
Objectives: Identify and preclinically characterize FGFR mutations with unknown functional impact.
Methods: We have identified a set of FGFR VUS based on their recurrence in the Catalogue Of Somatic Mutations In Cancer (COSMIC) or selected manually due to their positional and presumed functional importance. Cell lines carrying FGFR variants-of-unknown-significance (VUS) are generated using site-directed mutagenesis and retroviral transduction of NIH/3T3 fibroblasts. Potentially activating effects are tested by multiple in vitro assays: low-serum proliferation, soft-agar colony formation, and morphological transformation. The sensitivity of cell lines carrying activating mutants to clinically relevant tyrosine kinase inhibitors is assessed by IC50 determination in a viability assay.
Conclusions: Data generated by preclinical analyses contribute to overcoming the knowledge gap for FGFR VUS and can indicate potential treatment options. Additionally, we offer a comprehensive on-demand characterization service for VUS within the framework of the nNGM preclinical platform to both clinicians from nNGM sites and external clinicians.

Platform Biobanking and Data Management: Abstract No. 801 – 813

Abstract No. 801

Airway culturomics/functional microbiome platform

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Many microbiome studies have highlighted the relevance of commensals or secondary pathogen in disease progression using omics approaches. However, these studies are limited to a descriptive or correlation stage due to the lack of cultivable isolates to test their follow-up hypotheses. The objective of the platform is to offer extensive culture and isolation of microorganisms followed by in depth geno-phenotyping and functional characterization of the strains to test hypotheses made via metagenomic approach.
Samples are cultivated on 20+ media and under both anaerobic and aerobic condition to ensure a comprehensive coverage of all microbial ecological niches and the possibility to culture organisms from the rare biosphere. Single colonies isolated from culture media undergo species assignment and are stored in 20% glycerol stock media for biobanking purposes. In parallel, phenotypic resistance profile and genotype (WGS) are determined. Additional phenotyping, including virulence, metabolic and competition assays, are also performed to provide a thorough phenotypic profile for each isolate. To date, the platform has analyzed bio-material (sputum, BAL, tracheal secretion, throat swabs) from 52 patients (5 healthy control, 17 CF and 30 suspected pneumoniae) and cultured 632 strains from 120 different species.
The creation of a deep phenotyped biobank of isolates will allow *in vitro* and *in vivo* functional microbiome assays to explore the potential of commensals to reduce infection and/or inflammation and to modulate the microbiome to a more homeostatic state. The biobank will also be open to the scientific community so that researchers interested in specific organisms can request isolates of interest based on their phenotype or genotype.

Abstract No. 802

Improving ChipCytometry by automated cell recognition of airway samples using panoptic segmentation

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Background: The manual and automated segmentation of images containing cells from bronchoalveolar lavage (BAL) or induced sputum is challenging due to the high morphological complexity of cells. First attempts to automate image analysis using pixel-assigning semantic segmentation were able to distinguish cells from image background with an F_1-evaluation metric of 81.5%. The panoptic segmentation, however, not only enables clear assignments of pixels to cells but also their classification to different instances.
Method: Manually segmented ground truth data sets from transmitted light images of 207 BAL and 84 induced sputum were used to train and validate a panoptic segmentation algorithm. The quality of the segmentation was tested on an independent BAL data set of 131 images. Average precision (AP) and F_1 metrics were calculated to evaluate the closeness of predicted and ground truth results.
Results: Cells of the test data sets could be recognized well with AP and F_1 values of 92.9% and 88.0%. However, re-analysis of the training and validation data sets led to slightly reduced AP and F_1 values of 85.7% and 84.6% for BAL cells and 87.4% and 87.7% for induced sputum cells indicating an inconsistency of manually labeled ground truth data.
Conclusion: Panoptic segmentation allows a fast identification and classification of airway cells with acceptable average precision thereby improving results of manual and semantic segmentation. Inclusion of fluorescence images of cell surface stains will further enhance the cell recognition and pave the way for an automated analysis of cell subsets.

Abstract No. 803

DigitaLung: Digital auscultation system for differential diagnosis of lung diseases using machine learning

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The auscultation of lungs is a central part of medical examinations, but is subjected to a high variability depending on the age and experience of the physician. Given the transition to digitalization in medicine, and rapid improvements in signal processing afforded by modern AI techniques, there is a great opportunity to develop new technological solutions to the topic of differential diagnosis via auscultation. To meet this opportunity, we introduce the project DigitaLung, which aims to develop the necessary hardware, collect auscultation data and develop AI models to assist physicians in the diagnosis of lung disease.

The study seeks to recruit 2,000 patients with well-defined lung diseases as well as healthy controls, with the primary use case of differentiating bronchitis and pneumonia. During the current hardware development and data collection phase, the AI development team has been benchmarking various deep learning architectures such as convolutional neural networks and transformers on comparable public datasets (e.g., ICBHI 2017 Challenge). Complementary techniques, such as data augmentation and noise reduction have been tested. The preliminary results show that high accuracy (89 to 93%) is achievable for classifying disease types based on audio samples, but also highlight the challenges caused by imbalanced data and underrepresented disease types. Based on these results, the machine learning strategy can be adapted in anticipation of the completion of the data collection phase of the project. Although the DigitaLung project is in this early stage, these preliminary results indicate that the goal of AI-assisted auscultation is achievable in the near term.

Abstract No. 804

Preparation of a study protocol for the DZL Lung-Healthy Cohort

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The DZL runs a central data warehouse providing a constantly updated overview of overall 49.578 patients with 81.600 biospecimens and datasets available in DZL biobanks/databases. Systematic analyses of quality-assessed data showed that - although the DZL offers an impressive number of data sets and high-quality biospecimens from patients for scientific purposes - biospecimen and data from matched controls with comparable deep phenotyping are not sufficiently available. For translational research activities carried out within DZL, however, a comparison with appropriate controls, with a lung-healthy cohort (LHC), is indispensable. Existing cohorts are often not suitable for post-genomic methods due to insufficient phenotyping or low sample volume. We will develop a protocol for a LHC with adaptation to scientific needs and comparable deep phenotyping to our patients including a quality-controlled spectrum of biospecimen, functional and laboratory tests, and questionnaires aligned with existing clinical datasets.
The study aims to adjust the spectrum of biospecimen by a well-founded needs analysis among DAs and WGs with participation of data scientists. All controls will be asked for medical history data including the minimum data set, a guaranteed set of biospecimen, as well as functional tests and laboratory analyses to correlate experimental findings. Determination of indispensably required parameters, biospecimens and required number of volunteers will be task of a multi-stage query and coordination process including all target groups.
The LHC should allow state-of-the-art molecular technologies, such as single-cell sequencing, high-throughput omics analyses as well as imaging procedures. Initial pilot studies on a small scale are planned to test the developed protocol.

Abstract No. 805

Data driven validation of a digital solution to personalize anemia management in palliative care of lung carcinoma

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We developed a mechanism-based multiscale model that stratifies patients based on estimated patient specific parameters utilizing time-course data of Hb, CRP values and scheduled chemotherapy. The model was calibrated with individualized data from clinical trials with 417 lung carcinoma (LC) patients and 253 healthy subjects. The mathematical model can propose optimized personalized interventions for anemia in palliative LC patients, with minimal effective dose of ESAs and/or blood transfusions. The clinical application promises to lead to safer hemodynamic profiles, to increase the quality of life, to improve the response to palliative chemotherapy, and eventually reduce the risk of adverse events and mortality associated with the treatment. Accordingly, to advance the model towards clinical application we tested the accuracy of the hemodynamic predictions with datasets from clinical routine. We used the DZL Data Warehouse (DZL-DWH) platform, which allowed the search for available individualized patient data at the different clinical sites of the DZL, in a fast and systematic manner. 318 retrospective LC patients stage IV were identified and provided by DZL-DWH and implemented into our model prototype. A key advantage was that this large dataset of individualized patients was provided in a standardized manner and within the established DZL legal frame. This enabled us to analyze the prototype performance in real-world large clinical datasets, and to finalize a higher fidelity prototype. The developments that become possible due to the DZL-DWH significantly increase the efficiency of the investment of costly resources and improve the on-target development of standardization required in the final medical conceptualization before conducting a prospective clinical trial.

Abstract No. 806

Testing and implementation of a new temperature monitoring system for the Lung Biobank Heidelberg

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High quality biospecimens are the prerequisite for successful translational research. Preanalytical assessment is as important as controlled long-time storage of the samples. A temperature monitoring system is part of the new biobanking norm DIN EU ISO 20387 and therefore required for the long-time storage of samples. Here we report the testing of a newly implemented temperature monitoring system at Lung Biobank Heidelberg during the accreditation process in 2022. After physical implementation of the calibrated sensors into the freezers, the sensors were added to the virtual network. Alarms were generated simulating the loss of connection to the server, loss of connection to the sensors, a power failure or elevated temperatures. The generation of an audit trail was successfully established to monitor potential alarms. In summary, to put the new temperature monitoring system through its paces is essential to guarantee permanently controlled high quality samples that are stored over years.

Abstract No. 807

Migration of legacy data from the Heidelberg Lung Biobank

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Since the foundation of the Lung Biobank Heidelberg in 2001, different data storage systems were used over time including Excel, Access and StarLims databases. With the implementation of StarLims 12 as biobank laboratory and management system (LIMS) we planned to combine all available data resources within one common Lab system for an improved overview and handling of data / sample requests.
First of all, we developed the storage structure of the new target system (StarLims version 12). The data structure of the existing data bases had then to be transformed into a format compatible to the target system by means of an ETL upstream process.
We succeeded in a preliminary import of all existing biobank data. However, this process was accompanied by mapping losses during the transfer and the partial loss of metadata. The biggest challenge here was the altered storage system of biomaterial samples (i.e. 2d-barcoding, different aliquoting, box filling rules) whereas the maintained information on medical data could be transferred without major obstacles. Former biomaterial management data were found to be partly incompatible with the new data structure.
Data migration of legacy data is possible but losses of metadata will occur if hierarchical structures of source and target differ substantially. As a consequence, migrated legacy data have to be validated for completeness and correctness in the common management.

Abstract No. 808

Structural design for the integration of imaging data into the DZL Data Warehouse

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Thoracic CT scan is a major radiological tool and a gold standard for assessing various lung disorders. The integration of imaging data into the DZL Data Warehouse is an important goal in DZL 4.0 for provision of scientific secondary use. For this purpose, a common ontology that contains the essential information is required. Imaging data and associated clinical data in the DZL data warehouse will furthermore serve as an important resource for training of neural networks. In a first step we collected metadata from descriptions of relevant radiological information from experienced radiologists for each Disease Area (DA). This dataset has subsequently been expanded by online and literature information, focusing on professionally reviewed and evaluated data content (medical lexica, radiology encyclopedias, scientific articles, radiological reference books). The radiology information system (RIS) containing image descriptive data in a potentially evaluable form is an important additional source of metadata here.
Structured collections of radiological findings have so far been processed for selected DA's and need to be further refined, with others awaiting further processing. In a next step, the collected information is currently being structured graphically by Xmind. Due to the overlap in DA, mapping in a tree hierarchy and representation of multi-dimensionality is challenging.

Abstract No. 809

An integrated cell atlas of the lung in health and disease: current and future work

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Single-cell technologies have transformed our understanding of human tissues. Yet, studies typically capture only few donors and disagree on cell type definitions. Integrating many single-cell datasets can address these limitations of individual studies and capture the variability present in the population. Here, we present the integrated Human Lung Cell Atlas (HLCA), combining 49 datasets of the human respiratory system into a single atlas spanning over 2.4 million cells from 486 individuals. The HLCA presents a consensus cell-type re-annotation with matching marker genes, including annotations of rare and previously undescribed cell types. Leveraging the number and diversity of individuals in the HLCA, we identify gene modules that are associated with demographic covariates such as age, sex and BMI, as well as gene modules changing expression along the proximal-to-distal axis of the bronchial tree. Mapping new data to the HLCA enables rapid data annotation and interpretation. Using the HLCA as a reference for the study of disease, we identify shared cell states across multiple lung diseases, including SPP1+ profibrotic monocyte-derived macrophages in COVID-19, pulmonary fibrosis, and lung carcinoma. Overall, the HLCA serves as a pioneering example for the development and use of large-scale, cross- dataset organ atlases within the Human Cell Atlas.

Currently, we are preparing version 2 of the HLCA. This version will focus on combining single-cell data with other clinical information to better understand the link between cellular and organism-le phenotypes. Specifically, HLCA v.2 will focus on COPD, combining multi-omics (spatial, chromatin, protein and RNA) and clinical data (e.g. pulmonary function tests) of healthy and COPD

patients to complement HLCA v1.

Contribution of a Disease-spanning Working Group: Single Cell Analysis

Abstract No. 810

Establishment of precision cut lung slices as ex vivo model representing the tumor heterogeneity

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Despite of continuous developments of treatment options, lung cancer is still one of the most frequently diagnosed cancer worldwide and is the leading cause of cancer related death. Since previously used models such as animal models or cell cultures have certain limitations, there is a need for reliable and more predictive preclinical models to reduce translation failures and develop new therapeutic approaches.
Here, we demonstrate the establishment of the precision cut lung slices as an ex vivo model capable of representing the high intratumoral heterogeneity preserving the architecture, stroma, and cells of the tumor microenvironment in non-small cell lung cancer. First, together with the Institute of Pathology, a work flow was developed to generate formalin-fixed, paraffin-embedded PCLS as preparation for subsequent analyses as spatial analyses, multi immunofluorescence or immunohistochemical analyses. We optimised the thickness of the PCLS to allow siRNA infiltration in the entire depth of the PCLS. Furthermore, different lysis conditions were tested to optimise protein extraction for downstream analyses. PCLS dissociation with and without cryopreservation was applied to determine a potential loss of viability.
Our results demonstrate that the cultivation over four days showed only a low apoptosis level, that the PCLS can be successfully manipulated by i.e. siRNA transfection and that yielded protein amount per PCLS is sufficient for downstream analyses as immunoblot or mass spectrometry. Taken together, the PCLS can be used as a solid model for a variety of translational research studies and an alternative to 2D and 3D cell culture.

Abstract No. 811

Identifying drug repurposing options in scientific literature using natural language processing: The use of benzofuran-derivatives in CTEPH?

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The increase in scholarly output is resulting in an overwhelming volume of information. This study aimed at creating a software capable of identifying medication repurposing candidates through automated analysis of scientific literature.

Unknown links between medications and disorders are discovered using natural language processing (NLP) approaches such as term associations (word2vec). To demonstrate the software's capability, the search phrase *chronic thromboembolic pulmonary hypertension* (CTEPH), a rare illness with small literature corpus, is used.

First, clinical features semantically related to CTEPH are identified by sorting the CTEPH literature corpus by the information gain score (TF-IDF), a measure of a term's impact. These clinical features are used to identify associated diseases, and frequency of occurrences are quantified. The highest TF-IDF and counts were identified for *venous thrombosis* within the CTEPH corpus indicating significant semantic links between the diseases. Second, a list of prospective therapeutic repurposing candidates for CTEPH was generated by performing term associations on the CTEPH and venous thrombosis literature corpora. Benzofuran-derivatives were one of the highest-scoring candidates that were not previously referenced in the CTEPH literature. A structural search and *in silico* toxicological prediction highlighted amiodarone, an anti-arrhythmic medication. Finally, amiodarone reduced platelet aggregation by 68% ($p=0.02$) in response to ADP activation *in vitro*, demonstrating potential antithrombotic properties.

Automated analysis of scientific literature is an objective methodology considering the language employed to articulate a problem or objective, with the aim of disseminating knowledge across various scientific fields.

Abstract No. 812

Comorbidity extraction from clinical data sets §21 KHEntgG

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The main diagnosis of a patient is the central grouping concept of downstream clinical data collections such as biometric parameters, diagnostic procedures, therapies and clinical outcome. Nevertheless, additional comorbidities might have a strong impact on therapy decisions or the enrolling process for clinical studies. Thus, information on comorbidities is highly important for selection of patients and corresponding samples for scientific projects within DZL.
In 2019 we started a DZL-DWH project in order to investigate a possible relationship between lung cancer (LC) and asthma/allergy (AA). The first challenge was to define patient groups with main LC diagnosis with/without the AA comorbidity. To this end, we decided to develop a more general model leading to the linkage of an ICD coded main diagnosis and accompanying comorbidities. Based on a proposal of the German Medical Informatics Initiative (MII) we applied the hospital data sets according to § 21 KHEntgG. These data sets include a variety of tables related to reimbursement of diagnostic and therapeutic procedures and ICD coded diagnoses of patients. We determined the relationship between patient case numbers, therapeutic procedures (OPS), ICD codes and treatment intervals using talend software tool.
With help of the clinical experts we could define essential comorbidity groups relevant for disease areas within DZL. Finally, we aggregated individual ICD codes to comprehensive overarching classes of similar diagnoses i.e. E10-E14 Diabetes mellitus or E70-E90 metabolic disorders etc.
In summary, our solution will allow to identify patients and corresponding patient samples with a similar diagnostic phenotype for research purpose. This information will considerably enhance thereby the scientific value of the DZL-DWH.

Abstract No. 813

Implementation of TNM edition upgrade for malignant pleural mesothelioma within SAP i.s.h. med

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TNM upgrades change the classification as well as risk grouping of patients and should be considered in clinical routine, trial or scientific purposes. The documentation of pleural mesothelioma (PM) patients at the Thoraxklinik Heidelberg started according to UICC 5th Edition. With the upcoming 8th TNM Edition we decided to design a flexible model to achieve goals such as, new staging, re-staging and simplified technical implementation of new TNM upgrades in future within SAP i.s.h. med - hospital information system (HIS).
In a first step TNM and staging schemes of the revised UICC from 5th to 8th edition were analyzed and compared. Subsequently an overview table of TNM rules across all versions was created. Combining the table with the PM diagnosis date resulted in a sustainable technical solution for the upgrade of the TNM edition. In consequence, a new electronic form for registration of PM Patients has been developed.
In summary, the new electronic form allows the automatic re-classification and re-staging process according to the pattern of the current 8th TNM edition. Our solution ensures compliance of the uniform TNM staging data from all PM patients in our HIS independent of the date of diagnosis – including past, present and future cases. The performed work has contributed to the success in the accreditation of the Thoraxklinik Heidelberg as certificated pleural mesothelioma center. Last but not least, the PM patient data will be continuously updated with regard to the TNM Editions upgrade for scientific projects within DZL DWH.

Platform Imaging: Abstract No. 901 – 934

Abstract No. 901

Feasibility of perfusion-weighted phase-resolved functional lung (PREFUL) MRI with multi-echo ultrashort echo time sequence (PUTE)

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Introduction: Phase-resolved functional lung (PREFUL) MRI[1] is a free breathing 1H lung MRI postprocessing technique to gain dynamic ventilation and perfusion information from one data set[2,3]. To evaluate the influence of different echo times (TE) on perfusion parameters, a combination of PREFUL with a 2D multi-echo ultrashort echo time sequence (PUTE) was developed and perfusion parameters derived at different echo times were compared to each other and to PREFUL derived from a spoiled gradient echo sequence (SPGRE).

Methods: A 2D multi-echo UTE sequence was developed according to Triphan et al.[4]. All spokes were sorted according to their cardiac phase and dynamic image reconstruction was performed[5]. UTE and SPGRE scans were performed in free breathing for one healthy subject and one patient with pulmonary fibrosis with the following sequence parameters: UTE: TEs 0.07ms, 1.72ms, 3.37ms and 0.82ms, 2.47ms, 4.12ms TR 7.35ms/8.10ms, FoV 500x500mm^2, slice thickness (ST) 15mm. SPGRE: TE 0.82ms, TR 3ms, FoV 500x500mm^2, ST 15mm. Data sets were evaluated with PREFUL postprocessing. Perfusion-weighted maps (Q), and perfusion defect percentage (QDP) maps were compared.

Results: A similar visual appearance of perfusion maps derived with SPGRE-PREFUL and PUTE is shown for the healthy subject and the patient. Comparing PUTE QDP maps of the healthy subject, more perfusion defects can be seen for higher TEs. For the healthy subject, median perfusion-weighted values were highest at TE 0.07ms and 0.82ms. and for the fibrosis patient, a peak was reached at TE 0.82ms.

Conclusion: We could show the feasibility of PUTE with regional perfusion similarities on visual inspection compared to SPGRE-PREFUL. Further analysis in patient cohorts is necessary as well as the adjustment of PREFUL settings for PUTE.

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Abstract No. 902

Correlation between Functional Imaging and Morphological Features in a Mouse Model of Pulmonary Fibrosis

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Introduction: Limited therapeutic options and poorly understood pathogenesis stress the need of addressing IPF in research. The objective was to identify functional µCT-imaging features in Nedd4-2 mouse model of pulmonary fibrosis and by correlation with morphological features, assess the comparability of these approaches for differentiation of Nedd4-2 and wildtype.
Methods: Nedd4-2 mice were analyzed in 3D dynamic in vivo micro computed tomography (µCT) studies with prospective respiratory gating. Resulting image data sets were processed through VGStudio Max. Murine lungs were digitally segmented to assess lung volume and density, through gray value analysis, in four different respiratory states: inspiration, exspiration and two states in between. The correlation between functional µCT-imaging features, as mean gray value, aerated lung volume, relative total lung volume and relative aerated lung volume, with features from morphological assays, as a micro-radiological *in vivo* fibrosis score or histology parameters and murine pulmonary function parameters, as inspiratory capacity and compliance, were analyzed.
Results: In Nedd4-2 mice a higher lung density at inspiration and expiration ($p < 0.05$) and a higher reduction in aerated lung volume at expiration relative to inspiration ($p < 0.05$) were observed in comparison to wildtype. Inspiratory capacity and compliance were positively correlated with the relative aerated lung volume ($0.65 \leq r \leq 0.77$) and negatively correlated with the mean gray value ($-0.68 \leq r \leq -0.54$) during breathing cycle in Nedd4-2 mice.
Conclusion: Stand-alone assessment of dynamic *in vivo* µCT scan images promises to be of high relevance in future studies of mouse models of pulmonary fibrosis, as both functional and morphological features can be deduced.

Abstract No. 903

LuMaS: A robustness score for lung segmentation algorithms on CT images

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Research Question:
Lung segmentation (LS) is an essential step before automated lung image analysis. In practice, manual expert LS can be time-consuming and expensive. Therefor there are numerous LS algorithms available, which vary in performance on different lung pathologies. Our aim is to investigate if the quality and robustness of LS algorithms can be quantified objectively.

Objective:
We try to develop a robustness score for LS algorithms based just on the imaging properties of CT using in- and exhale scans.

Methods:
Hounsfield units (HU) in a 3D CT can be used to assess different tissue types By assuming a linear relationship of HU values to mass density in the lung window, the mass of each voxel can be calculated. As the mass of lung tissue is constant in inhale and exhale CT scans of a patient, a perfect LS algorithm should yield the same lung mass for both scans. For evaluation, we defined the Lung Mass Score (LuMaS) as the relative deviation between the lung mass calculated from inhale and exhale image. We investigated errors that occurred due to the discrepancy between the manual expert LS and the theoretical prediction of 0%.

Results:
Manual expert LS and deep learning-based yield to a mean LuMaS of 5%, classical approaches to 15% (Thresholding) and 10% (Watershed).
An error analysis showed that perfusion of the lungs between inhale and exhale may influence lung weight change during respiration. Further, including large HU values in the segmentation resulted in a decrease in the sensitivity of LuMaS. In addition, a difference of LuMaS was found for different manual expert LS conventions.

Conculsion:
LuMaS quantifies the robustness of LS algorithms, although its limitations must be taken into consideration.

Abstract No. 904

Comparing Automated Radiomics Pipelines: A Performance Evaluation on Open Source Multi-Modal Datasets

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Radiomics is a flourishing field in medical imaging that aims to extract quantitative features from images for use in clinical decision-making. In our study, we aimed to improve the automated configuration concept of autoradiomics by incorporating state-of-the-art feature selection techniques and optimizing random forests for better performance. Radiomics analysis involves several complex steps, which can be time-consuming and labor-intensive. To help automate these steps, the Autoradiomics tool [1] and WORC [2] were developed. We developed a radiomics pipeline that uses Pyradiomics for feature extraction, feature variation and correlation filtering, sequential feature selection, and grid search optimization of a random forest classifier. Our study involved the application of our radiomics pipeline to six open-source datasets [3] including medical CT and MRI images from different entities and classification problems, designed to facilitate and validate radiomics research. We compared the performance of our pipeline to published results from Autoradiomics and WORC. We were able to either outperform or achieve similar results in 5 out of 6 datasets. On average, our performance difference to Autoradiomics across all datasets was +0.03 (+-0.14), and to WORC was +0.09 (+-0.07) Test AUROC. Our study highlights the potential of automated radiomics pipelines and the gain in performance based on configuration, leading to improved workflow design, simplicity, and accessibility.

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Abstract No. 905

Influence of comorbidities and the severity of pneumonic infiltrates on the outcome of COVID-19 positive patients: a CT-based multi-center study

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Purpose
The aim of this retrospective, five-center study was to analyse which pre-existing comorbidities influence the clinical course of patients suffering from COVID-19 pneumonia. Furthermore, the severity of pneumonic infiltrates detected in chest CT scans was quantified and correlated with the patients´ outcome.
Materials and Methods
Patients hospitalized with COVID-19 pneumonia between 2020 and 2021 who underwent a chest CT scan were included into this study. Clinical endpoints defined were mortality, stay at the intensive care unit (ICU) and intubation. Cardiac, chronic (obstructive) pulmonary, kidney, liver and malignant disease, immunosuppression, diabetes type 2, arterial hypertension and obesity were comorbidities analysed. CT scans of the whole lung with a slice thickness of a maximum of 1.5 mm were assessed for ground-glass-opacity index, fibrosis index (FIBI), mean lung density, 15th and 75th percentile (Perc15, Perc75) using an in-house written software (YACTA). Logistic regression models were used for data analysis.
Results
A total of 259 patients (67% male, mean age 63 years) were included in the study. Patients mortality was significantly increased by high age ($p<0.001$), when suffering from chronic cardiac disease ($p=0.042$) and elevated FIBI ($p<0.001$). The risk for ICU stays decreased for female sex ($p=0.006$) and increased for elevated FIBI ($p<0.001$). Increased FIBI ($p<0.001$), decreased Perc15 ($p=0.001$) and pre-existing cardiac disease ($p=0.01$) rose the risk for intubation.
Conclusion
In this study cohort chronic cardiac disease significantly increased patients´ mortality and need for intubation. FIBI was the most reliable imaging marker to estimate patient outcome.

Abstract No. 906

Modeling the normal lung: Identifying COPD regions as anomalies using deep learning contrastive representations on chest CT

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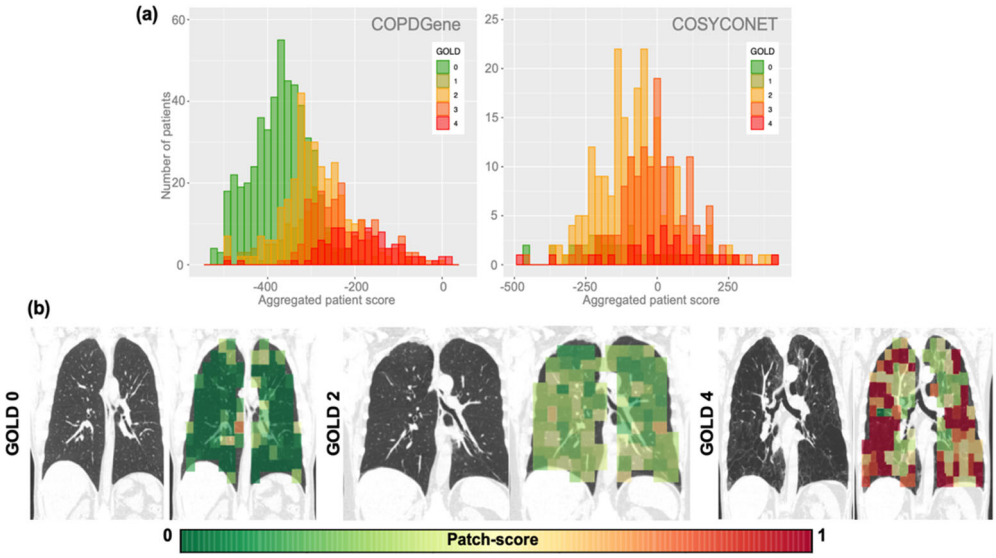
Background: Classification of heterogeneous diseases is challenging due to their complexity, variability of symptoms and imaging findings. Chronic Obstructive Pulmonary Disease (COPD) is a prime example, being underdiagnosed despite being the 3rd leading cause of death. Its sparse, diffuse and heterogeneous appearance on CT challenges supervised binary classification. **Purpose:** We reformulate COPD binary classification as an anomaly detection task: heterogeneous pathological regions are detected as Out-of-Distribution (OOD) from normal homogeneous lung regions. **Methods:** Representations of unlabeled lung regions are learned by a self-supervised contrastive pretext model, potentially capturing specific characteristics of diseased and healthy unlabeled regions. Then, a generative model learns the distribution of healthy representations and identifies abnormalities (stemming from COPD) as deviations. Patient-level scores are obtained by aggregating region OOD scores. **Results:** Our method achieves the best performance on two public datasets, with an increase of 8.2% and 7.7% in terms of AUC compared to the previous supervised state-of-the-art. Additionally, it yields well-interpretable spatial anomaly maps and patient-level scores which we show to be of additional value in identifying individuals in the early stage of progression. **Conclusion:** Anomaly detection is a powerful way of tackling COPD classification and is potentially applicable to other diffuse lung diseases.



(a) Patient distribution by mean aggregated scores, colored by the function risk score (GOLD), for COPDGene and COSYCONET. (b) Coronal view of the score map on three subjects with different degrees of severity. Min-max normalization was applied to patch scores, corresponding to the 5th - 95th percentiles of the corresponding testset.

Abstract No. 907

Magnetic Resonance Imaging Detects Delayed Perfusion Inside Perfusion Defects in Patients with Cystic Fibrosis

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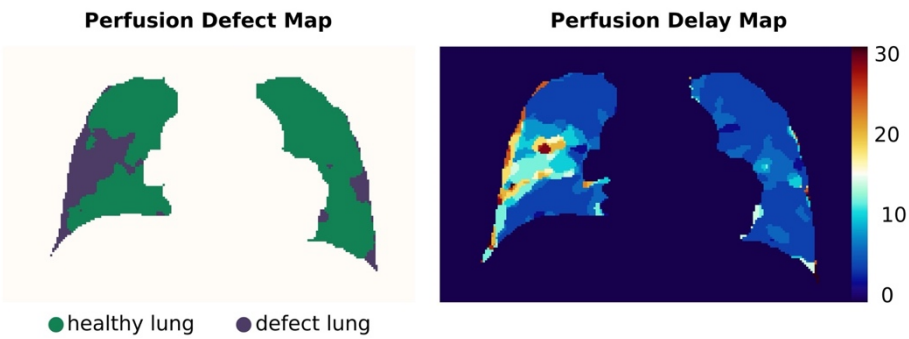
Objectives: Dynamic Contrast Enhanced Perfusion MRI (DCE-MRI) detects pulmonary perfusion abnormalities in patients with cystic fibrosis (CF). Little is known about the alterations of bronchial arterial inflow from the systemic circulation. We hypothesized that in perfusion defect areas a delayed perfusion from bronchial arteries may be observed. **Methods:** Morpho-functional MRI from 50 CF-patients were included. The lung was segmented on a 3D GRE and registered onto DCE-MRI. An automatically placed ROI in the pulmonary artery was used to calculate the arterial input function (AIF), which was then used to quantify pulmonary blood flow and mean transit time. Perfusion defects were classified using Otsu's method for clustering the R(t) maps at the time of maximum contrast enhancement, to quantify defects in percent (QDP). Perfusion delay maps were calculated by cross-correlation between AIF and each voxel of the subtraction images. Quantitative perfusion parameters were compared in areas with normal perfusion vs. perfusion defects. **Results:** Perfusion in the healthy areas occurs mostly at the same time, whereas delayed perfusion is visible in the defect areas (see *Figure 1*). **Conclusion:** Lung areas with perfusion defects in the pulmonary arterial phase show delayed perfusion in the systemic arterial phase in patients with CF, likely through bronchial arteries. Further studies may investigate the correlation of delayed perfusion with bronchial artery dilatation in advanced CF.



Perfusion defect map (left) and perfusion delay map (right) of a 14-year-old CF patient. Healthy areas show similar small perfusion delays, whereas defect areas show a heterogenous delayed perfusion.

Abstract No. 908

Electron Microscopy within the DZL

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As Research Core Unit of the Hannover Medical School, we are part of the DZL-site BREATH and execute electron microscopic imaging and analysis in lung research. Our technical portfolio ranges from classical TEM and SEM imaging over correlative applications of light- and electron microscopy (CLEM) to various 3D reconstruction techniques including serial block-face SEM, focused ion beam SEM and electron tomography. We are constantly engaged in optimization and adaption of preparation and imaging conditions for each individual scientific approach.
Here, our current scientific collaborations within the DZL are presented, for which different EM techniques are being used to gain subcellular structural insights:
Cilia in cell culture as well as human samples of patients suffering from primary ciliary dyskinesia were analyzed systematically concerning the alteration of cytoskeletal elements. A clear correlation of defined structural defects and the disease was shown, reinforcing the diagnostic potential of EM here.
In a 3D-approach, foldings of the alveolar basal lamina were modelled based on serial block-face SEM, giving rise to a better understanding in general mechanics and sterical processes in the flow of in- and expiration.
A new method to massively increase the 3D field-of-view for targeting a certain region in a piece of tissue was recently established by us. This approach named “Volume-CLEM” was applied to image human fibrotic lung tissue in a correlative light- and electron-microscopic workflow in three dimensions, allowing to zoom in a targeted manner from a macroscopic range down to single cells and subcellular components.
Exemplified on these and further approaches we demonstrate here the extensive scope of application of electron microscopy at the DZL site Hannover.

Abstract No. 909

Predicting Mutational Status of Non-Small Cell Lung Cancer: A Radiomics and Deep Learning Approach

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Motivation: Assessing tumor mutational status is essential for targeted treatment approaches in advanced-stage lung cancers. However, given tumor heterogeneity, obtaining a representative biopsy for every tumor entity is not always feasible. Recent research indicates that image computing approaches may enable assessment of tumor mutations from imaging alone, informing on treatment options when biopsy of every mass is not feasible, known as virtual biopsy. This study aims to develop virtual biopsy for advanced NSCLC, comparing radiomics and deep learning approaches.
Methods: We retrospectively collected and annotated CT image data from 359 patients with NSCLC, carrying either ALK (n=45), EGFR (n=241), or none of these mutations (n=73). Radiomics features were extracted solely tumor-based, and extended with clinical features to train Random Forest, XGBoost and SVM classifiers to predict mutational status. For comparing prediction performance, we also trained image classification DenseNets on the full image volumes.
Results: Radiomics features alone yielded a classification accuracy of 0.51, increasing to 0.59 when combined with clinical features using Random Forest. Comparatively, multiclass classification of full image volumes with DenseNet resulted in an accuracy of 0.50, and One-vs-All DenseNet classification did not improve these results.
Conclusions: Deriving virtual biopsy for NSCLC-mutation prediction based on clinical CT image data alone does not seem feasible yet, despite employing different ML- and DL-models. However, inclusion of clinical features had a larger impact than model tuning, offering avenues for improvement. Therefore, further investigation using larger receptive fields is required when aiming at extracting robust imaging markers in the future.

Abstract No. 910

Improved image quality in dynamic OCT imaging of airway and lung tissue by reduced imaging time and machine learning based data evaluation

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Endoscopic morphological evaluation of lung tissue is a first-line diagnostic tool. An endoscope-compatible, contrast agent independent imaging technique that can capture the cellular morphology in vivo could expand endoscope-based diagnostic capabilities and enhance research of disease-related morphological changes.

Dynamic Optical Coherence Tomography (dOCT) combines the advantages of high-resolution tomographic imaging with cell-specific contrast. By acquiring time series and subsequent pixelwise Fourier Transformation, a high dimensional representation of signal fluctuations, created by subcellular movement, is achieved. Binning of the frequencies creates an RGB image. Thus, ex vivo, high-resolution images of airways can be generated, as we have previously shown. However, global movements can lead to artifacts and currently hamper in vivo application.

Therefore, the influence of time series length on motion artifacts and image quality was evaluated on data generated with an endoscope compatible setup on freshly excised surgical human lung material.
When using shorter time series, the RGB bins need to be adjusted. For this, we implemented the Neural-gas algorithm. It generates the highest contrast between spectral key points and rapidly converged. Both with algorithmic and manual binning, the reduction of the time series from 150 to 6 frames, with recording times of 1.3 s to 50 ms respectively, generated images that largely still contained all important information about the tissue structures.

The shortened acquisition time is a very important step for the in vivo application of dOCT. In addition, since the algorithm required no a priori knowledge, an autonomous analysis is possible, enabling a large throughput or even real time diagnosis.

Abstract No. 911

Value of Photon-Counting CT iodine-maps for differential diagnosis of parenchymal lung diseases: An observational study

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Purpose: The purpose of this study was to investigate whether iodine maps can be used to differentiate parenchymal disease with increased lung attenuation.
Methods and materials: 325 patients were scanned with a photon-counting CT (PCCT),using four scan protocols, all with lung parenchymal contrast. Lesions were classified into three basic patterns (consolidation, ground-glass opacities (GGO), and reticular patterns), each with several subcategories. Lesion classification was performed by 2 of 3 radiologists who were blinded to the diagnosis using a 5-point Likert scale. Only contrast-enhanced images were available for the first reading. After a delay of at least one week, a second reading was performed now including the iodine map and virtual non-contrast images.
Results: There were 206 lesions found with a confirmed diagnosis (83 consolidations, 72 GGO, and 51 reticular).There was a significant improvement in the Likert score for all three basic patterns (consolidations: 3.3 vs. 3.9, GGO: 3.4 vs. 4.1, and reticular: 3.6 vs. 4.41, $p < 0.001$).The iodine map was always helpful in the differential diagnosis of consolidations. There was no worsening of the score after the inclusion of the iodine map. However, there was a downgrading of the score for GGO and reticular pattern in three and one cases, respectively. The downgrading occurred for morphologically uncertain GGO findings (3) and atelectasis (1) with inhomogeneous iodine uptake. In 29 lesions, the classification was changed when the iodine maps were included in the evaluation.
Conclusions: PCD-CT allows the production of iodine maps of high quality, that improve diagnostic confidence in parenchymal pathologies with increased attenuation and facilitate diagnosis.

Abstract No. 912

Diffusion of ^{129}Xe in the lung: airspace size heterogeneity and airway connectivity assessed by filter exchange MR imaging (FEXI)

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Introduction: Diffusion of ^{129}Xe in an imaging voxel in the lung may be described by a distribution of diffusion coefficients (ADCs) due to airspace size heterogeneity.[1] The distribution is expected to differ between COPD/emphysema phenotypes. Purpose of this work was to develop a ^{129}Xe FEXI pulse sequence, probing airspace heterogeneity in COPD patients and healthy volunteers. **Methods:** FEXI[2] with some modifications (fast spiral readouts[3] and low-flip angle excitations) was implemented, see the sequence diagram. Whole-lung ADC was calculated and the model function $ADC(t)=ADC_0(1-a\exp(-Rt))$ fit to the data. **Results:** Four healthy volunteers and five COPD patients were included. ADC_0 was increased in COPD patients compared to healthy volunteers, p=0.016, as was R, p=0.032. a/R weakly correlated with ADC_0, r=0.33/0.57. **Discussion/Conclusion:** Probing heterogeneity and connectivity of lung airspaces is feasible using FEXI. Airspace enlargement reflected by increased ADC_0 in COPD may be associated with variable reduction a after filter application, hence tissue heterogeneity. R may reflect diffusional screening,[4] which is reduced in emphysema,[5] likely leading to faster recovery. In conclusion, ^{129}Xe FEXI MRI provides new biomarkers for COPD phenotyping.

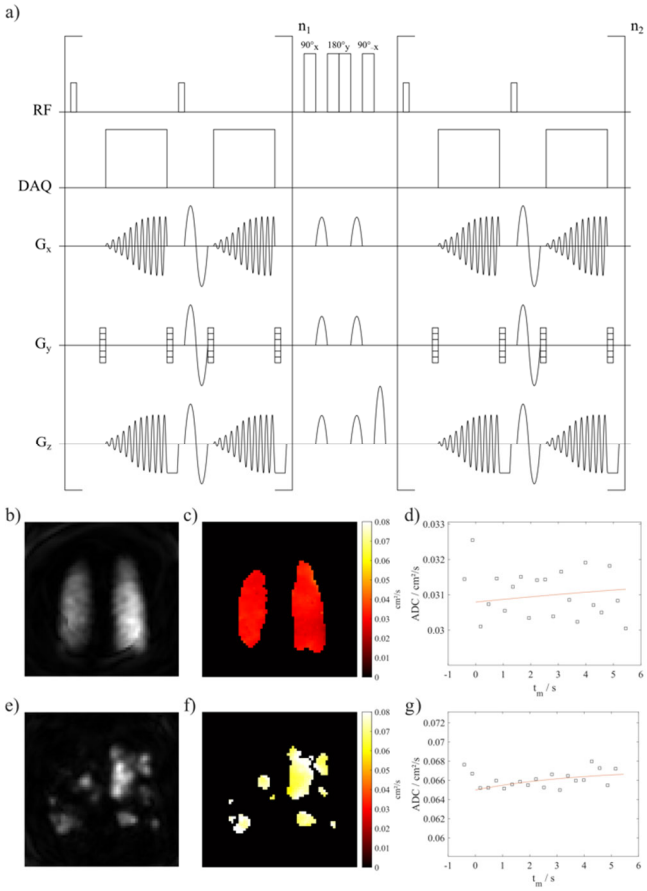

Figure 1. (a) Sequence diagram. ADC is measured repeatedly before and after saturating fast-diffusing spins. Representative 129Xe images (b, e), ADC maps (c, f) and whole-lung ADC dynamics (d, g) for a healthy subject and a COPD patient. Only small/slow changes of ADC are observed in the healthy subject with more pronounced changes in COPD.

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Abstract No. 913

Feasibility and Reproducibility of Ventilation and Perfusion MRI at a 0.35 T MR-Linac in Healthy Volunteers and Lung Cancer Patients

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Objective: MR-Linacs allow highly accurate radiotherapeutic treatment of lung cancer patients. We aim at demonstrating the feasibility and potential of Non-uniform Fourier Decomposition (NuFD), a non-contrast enhanced free-breathing technique to assess lung ventilation (V) and perfusion (Q), in 12 subjects at a 0.35 T MR-Linac. To improve V-map reproducibility, two normalization strategies are proposed.
Methods: 10 healthy volunteers were repeatedly scanned at a 0.35 T MR-Linac (MRIdian, ViewRay Inc., Cleveland, Ohio) using an optimized balanced steady-state free precession sequence: flip angle=70°, TR/TE=2.42/1.02 ms, FOV=500×500×20 mm^3, matrix=128×128×1, frame rate=3.68 images/s, total TA=1.1 min. Image series (240 images) of two coronal slices were acquired in normal, deep and shallow free-breathing with in-/outside scanner breaks for each volunteer and one slice at tumor position for 2 lung cancer patients after each treatment. NuFD was performed pixel-wise to generate V- and Q-maps. For intra-volunteer V-map reproducibility, a normalization factor was defined based on the linear correlation of V-signal and diaphragm position. The second strategy normalized the V-maps with the average V-signal within a selected ROI.
Results: NuFD was successfully applied to all subjects' scans. Median deviations between corrected and reference V-maps of <10% were obtained for both methods for all volunteers compared to 30% for uncorrected maps. Application of the ROI-based method on the patient data showed improving V in the tumor-surrounding tissue.
Conclusion: Applying normalization methods clearly improves the V-map reproducibility. First longitudinal V-assessment over treatment courses shows promising results for the use of NuFD as treatment response monitoring tool in the clinic.

Abstract No. 914

Validation of functional free-breathing 3D phase-resolved functional lung (PREFUL) MRI with dynamic ^{19}F ventilation MRI in patients with obstructive lung disease and healthy volunteers

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Purpose: To validate ventilation parameter maps calculated using 3D phase-resolved functional lung (PREFUL) MRI with ^{19}F wash-in MRI in patients with obstructive lung disease and healthy volunteers.

Materials and Methods: 29 patients with chronic obstructive lung disease and 13 healthy volunteers underwent free breathing 3D PREFUL MRI [1] and ^{19}F wash-in MRI [2]. For 3D PREFUL, static regional ventilation (RVent) and dynamic flow-volume based cross-correlation metric (FVL-CM) were calculated. For both parameters, ventilation defect percentage (VDP) values and ventilation defect (VD) maps (including combination of both parameters ($VDP_{Combined}$)) were determined. For ^{19}F MRI, images from eight consecutive breaths under volume-controlled inhalation of perfluoropropane were acquired. Dynamic time-to-fill (TTF) and wash-in parameters were extracted. For each breath-hold, VD map and VDP values were calculated and considered for the comparison with 3D PREFUL. All parameters of both techniques were compared using Spearman correlation coefficient. The differences between VDP values were compared using Bland-Altman analysis. For regional comparison of VD maps, spatial overlap and Sørensen-Dice coefficients were computed.

Results: On a global level, all 3D PREFUL VDP maps were significantly correlated to all VDP measures of ^{19}F ventilation imaging, except for the first breath (all $r > 0.48$; all $P \leq 0.0016$). Regarding regional comparison, the best overlap of defect areas was between 3D PREFUL $VD_{Combined}$ maps and ^{19}F derived VD_{TTF} and VD of first and second breath (Sørensen-Dice coefficients ≥ 0.28).

Conclusion: 3D PREFUL MRI showed good correlation with ^{19}F MRI on a global level and decent spatial correspondence of ventilation defects on a regional level.

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Abstract No. 915

Location specific pathology analysis of the monopodial pulmonary vasculature in a rabbit model of bronchopulmonary dysplasia

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The mammalian pulmonary vasculature consists of functionally and morphologically heterogeneous compartments. When comparing sets of lungs with different experimental backgrounds or treatments, e.g. disease models or therapeutic interventions, local changes may be masked by the overall heterogeneity of the organ structure. Therefore, alterations taking place only in a sub compartment may not be detectable by global analysis. In the monopodial lung, which is found in the commonly used laboratory animals, the characterization of distinct vessel groups is difficult, due to the asymmetrical branching pattern. Here, a previously established method to classify segments of the monopodial pulmonary arterial tree into homogeneous groups [1] was employed. To test its suitability in an experimental setting, the method was applied to a hyperoxia (HYX, ≥95% oxygen) rabbit model of bronchopulmonary dysplasia and a normoxic control group (NOX, 21% oxygen). Using this approach, it was possible to identify morphological differences between the HYX animals and the NOX group. The method allowed pinpointing of globally visible differences to specific lung regions. Furthermore, local changes in single compartments, that would not have been identifiable in an unfocused analysis of the whole dataset, could be identified. HYX animals exhibited a smaller arterial lumen as well as a higher number of smooth muscle cell layers in the distal vessels, compared to the NOX group. The wall thickness of the proximal arteries was greater in the HYX animals. In conclusion, the method described here achieves a higher precision in morphological studies of lung disease models, compared to a common, global analysis approach.

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Abstract No. 916

Deep learning-based air trapping quantification analyzing structure-function relationships using paired inspiratory-expiratory ultra-low dose CT

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Objective: Air trapping (AT) is a common finding in early cystic fibrosis (CF) lung disease detectable on computed tomography (CT). Conventional methods for AT quantification are density-based thus influenced by CT dose. Since dose reduction is one of the main objectives in CT, we investigated its influence on AT quantification by comparing low dose (LD) with ultra-low dose (ULD) CT. **Methods:** Same-session paired spirometry-guided inspiratory-expiratory CT scans of 52 CF subjects were acquired at LD (CTDI 1.22±0.56 mGy) and ULD (CTDI 0.22±0.05 mGy). Multiple breath washout (MBW) was performed to obtain the lung clearance index (LCI). 3-D deformable image registration was applied to spatially align the inspiration with the expiration. 2-D patches were created of the expiration, the corresponding registered inspiration and the radiographic ground truth. A densely connected convolutional neural network (DenseNet) was trained to segment AT. Grid search was performed to examine the usage of one or two input channels and the training on one or both scan protocols. **Results:** A good AT segmentation was achieved and AT percentages correlated strongly with the MBW results for LD and ULD (LD:R=0.76,p<0.001;ULD:R=0.78,p<0.001) (Fig.1). A strong ULD-LD correlation (R=0.96,p<0.001) and small ULD-LD differences were observed. **Conclusion:** Despite the 82% dose reduction in ULDCT, results suggest that the ULD scan protocol allows for comparable AT quantification and training is sufficient on LD only.

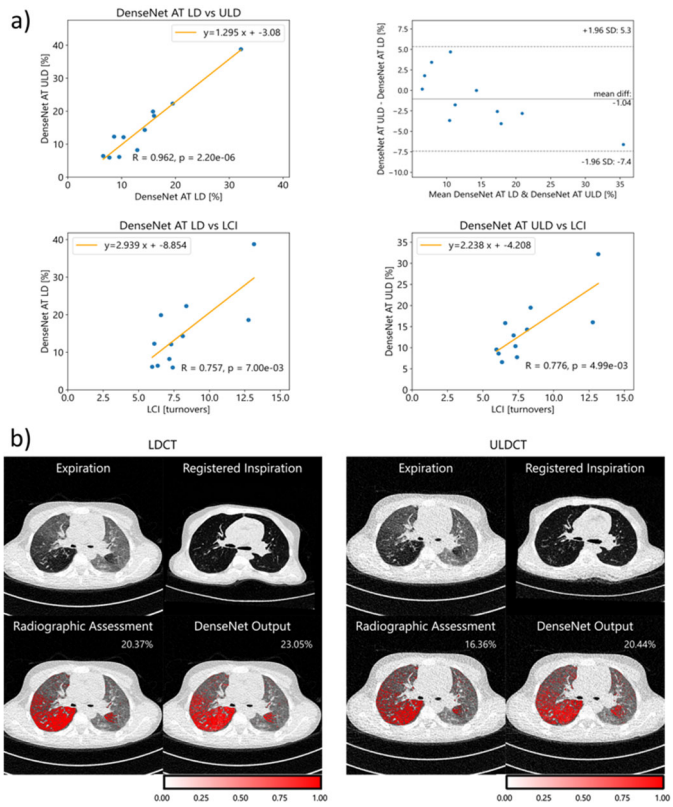

Fig.1: Best model results. a) Correlations and Bland-Altman analysis. Strong correlation with LCI (LD:R=0.76,p<0.001;ULD:R=0.78,p<0.001). Strong ULD-LD correlation (R=0.96,p<0.001). Small mean ULD-LD difference of −1.04±3.25%. b) Inputs, ground truth and the output probability map for a LD and ULD CT scan. The AT percentage is displayed in white.

Abstract No. 917

Quantification and Optimization of Image Registration Algorithms for Fourier Decomposition (FD) Lung Magnetic Resonance Imaging (MRI)

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Background: FD MRI techniques use a pixel-wise frequency analysis of dynamically acquired data sets to separate the signal changes arising from the respiratory and cardiac cycles. This requires the application of a retrospective image registration approach to correct for the motion of the pulmonary structure. The quality of FD MRI thus strongly depends on the performance of the image registration.
Purpose: To develop metrics that objectively quantify the remaining motion after image registration and to optimize and compare the registration tools Elastix [1] and ANTs [2] based on these metrics.
Methods: Free breathing lung MRI scans of seven healthy volunteers (age: 38±14 years) and three lung cancer patients (age: 61±3 years) were acquired on a 0.35 T MR-Linac using a balanced Steady State Free Precession sequence. The tools Elastix and ANTs were quantitatively assessed by means of independent methods, estimating the motion reduction in the registered images by judging the stability of the diaphragm, the lung margin, and the position of high contrast blood vessels. The tools were optimized for registration outcome and computational time with regard to their registration parameters, the application of a mask, and the choice of the reference frame.
Results: The remaining motion with the Elastix registration was reduced from 36.3 % to 33.1 % with an optimized parameter set, reducing the computational time from 677.5 s to 417.4 s. The remaining motion for ANTs was reduced from 32.2 % to 28.4 %, accepting an increase in computational time from 250.9 s to 321.5 s.
Conclusion: The registration outcome of both tools was improved. ANTs is the more reliable and faster implementation.

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Abstract No. 918

Quantification of cell-cell contact between alveolar epithelial type II cell and fibroblasts in human lung

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Interaction between alveolar epithelia type II cells (AT2s) und fibroblasts seems to be involved in the alveolar homeostasis. Reduction of cell-cell contact was observed in human lung emphysema and bleomycin induced lung injury in mouse model. Previous studies suggested different forms of such contacts based on transmission electron microscopy (TEM) and volume data, however with unsatisfiable resolution in z-axis. To investigate the ultrastructure of the cell-cell contact, we used one continuous image stack derived from focused ion beam scanning electron microscopy (FIB-SEM) as a template for the manual segmentation to generate a 3D model. The image stacks obtained from serial block face scanning electron microscopy (SBF-SEM) were used to determine the number of cell-cell contact. The resulting 3D model shows the precise ultrastructure of contact between AT2s and fibroblasts consisting of foot processes of AT2, hole in the basal lamina and cytoplasmic extension of fibroblast. Various forms and complexity of contact were found. Interestingly, the foot processes of AT2s generally appear as clusters, which scatter over the basal surface of AT2s. Some of these clusters coil underneath the intact basal lamina making no contact to fibroblasts. The number of cell-cell contact per AT2 ranges between zero to twelve, with the majority of cells having zero to five contacts per cell. For this ongoing study, we plan to increase sample number and establish correlative light and electron microscopy to identify previously described intermediate AT2s, whose morphology and interaction with fibroblasts will be consequently investigated in context of lung fibrosis.

Abstract No. 919

A multiplexed imaging platform for perturbations and drug development in a human fibrosis model

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Novel methodologies and protocols for multiplexing of tissue complexity are currently developed, facilitating the identification of new cell types, disease-associated cell states, and intercellular communication. Here, we successfully developed an imaging analysis pipeline, using FFPE sections of punched human precision-cut lung slices (hPCLS) treated with a pro-fibrotic cocktail (FC) together with Nintedanib or a novel antifibrotic compound series (N23Ps). These FFPE sections were immunofluorescently stained for protein markers of various fibrosis-relevant cell-types which were previously identified by single cell or single nuclei techniques. Next, we applied high-throughput imaging using automatized slide scanning and applied single cell segmentation analysis by machine learning pixel classification. In combination with a time-resolved analysis at 12, 24 and 48 hours after FC treatment in hPCLS, we could provide solid evidence for a model that supports AT2 cell differentiation towards aberrant KRT17+/KRT5- basaloid cells in human lung fibrogenesis. Finally, we further expanded this pipeline and successfully applied Iterative indirect immunofluorescence imaging (4i) to FFPE sections derived from punched hPCLS. Iterative indirect immunofluorescence imaging (4i) is a recently established method achieving multiplexed protein staining within the same biological sample. Prospectively, human tissue microarrays (TMAs) containing multiple patient lung tissue samples on one single slide will increase the throughput, and by combining automatized and software-based analysis tools, this platform will allow for the large-scale validation of multi-omics findings for lung health and disease, and for the elucidation of mode-of-actions of novel therapeutics.

Abstract No. 920

Analysis of the alveolar shape in 3D

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Mechanical forces affect the alveolar shape, depending on location and tissue composition, and vary during the respiratory cycle. This study performs alveolar morphomics in different lobes of human lungs using models generated from 3D microCT images. Cylindrical tissue samples (1.6 cm × 2 cm) were extracted from two nontransplantable donor lungs (one ex-smoker and one smoker, 3 samples per subject) that were air-inflated and frozen solid in liquid nitrogen vapor and scanned with microCT (11 µm/voxel). Within representative cubic regions of interest (5.5 mm edge length), alveoli were segmented to produce corresponding 3D models from which quantitative data were obtained. The surface of segmented alveoli (n_alv_total = 23,587) was divided into individual planar surfaces (facets) and angles between facet normals were calculated. Moreover, the number of neighboring alveoli was estimated for every alveolus. In this study, we examined intraindividual differences in alveolar morphology, which were reproducible in the lungs of two subjects. The main aspects are higher mean alveolar volumes 6.64×10^6 and 6.63×10^6 µm^3 vs. 5.78×10^6 and 6.29×10^6 µm^3) and surface sizes (0.19 and 0.18 mm^2 vs. 0.17 mm^2 in both lower lobes) in both upper lung lobes compared with the lower lobes. An increasing number of facets from top to bottom (12 and 14 in the upper lobes; 14 and 15 in the lower lobes), as well as a decreasing number of alveolar neighbors (9 and 8 in the upper; 8 and 7 in the lower lobes) from the upper lobes to the lower lobes were observed. By using this new approach of analyzing alveolar 3D data, which enables the estimation of facet, neighbor, and shape characteristics, we have established the baseline measures for in-depth studies of mechanical conditions and morphology.

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Abstract No. 921

Exploring the potential of Explainable Artificial Intelligence in Deep Learning for Lung Changes in Cystic Fibrosis Patients

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Introduction: Explainable Artificial Intelligence (XAI) algorithms have been developed to increase the transparency of deep learning models. They help to understand and interpret predictions [1]. In this study, we investigate the potential of XAI for the classification of lung perfusion defects and mucus plugging in cystic fibrosis (CF) patients on magnetic resonance imaging (MRI) [2].
Methods: We used a variety of algorithms, including integrated gradients and grad cam, on top of an existing deep learning-based classification pipeline. A total of six XAI algorithms were tested. Performance was evaluated by analysing the highlighted areas relevant for classification. The areas were compared to ground truth information and previously acquired radiological knowledge.
Results: Our results show that the areas relevant for classification were accurately highlighted in the majority of the cases, highlighting the potential of deep learning for the classification of lung changes in CF patients. Incorporating explicable concepts with deep learning could improve clinician confidence in deep learning, facilitating the adoption of more diagnostic decision support systems.
Discussion: However, the application of XAI algorithms to complex medical problems and custom neural network architectures remains challenging [3]. Future work will focus on the development and refinement of explicable algorithms that can effectively address these challenges. Ultimately, diagnostic confidence and the implementation of more effective decision support systems in clinical settings could be enhanced by integrating explicable concepts with deep learning.

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Abstract No. 922

Combined assessment of regional pulmonary morphology and function: Feasibility in a clinical setting with photon-counting CT

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Purpose

To report initial results using a modified chest CT protocol and photon counting CT (PCCT) for comprehensive analysis of pulmonary vasculature, perfusion, ventilation and morphology in a single examination.

Methods

The study group consisted of consecutively included patients with diffuse interstitial lung disease, CTEPH and long-Covid. After administration of iv contrast the inspiratory scan was triggered in the ascending aorta, followed by an expiratory scan after a delay of 5 minutes. The parameters calculated included maps of regional ventilation, perfusion, late enhancement, and CT angiography (CTA). Mean intravascular contrast in the mediastinal vessels as well as radiation dose were determined. Mean values of lung volumes, attenuation, ventilation, perfusion and late enhancement were tested for significant differences in subgroups of patients with different lung pathology.

Results

In 85% of 196 consecutively included patients, all parameters could be acquired. In the inspiratory scan, mean density was 325 HU in the pulmonary trunk, 260 HU in the left atrium and 252 HU in the ascending aorta. The mean dose length product (DLP) was 226.81 mGy*cm, and the mean computed tomography dose index (CTDI) was 4.29 mGy for the complete protocol. Significant differences between the subgroups were found for all assessed parameters. Visual inspection of the additional functional imaging maps revealed VQ mismatch, detection of pathologically increased ventilation associated with interstitial lung disease or increased perfusion in long-COVID.

Conclusion

The proposed PCCT protocol allows for a dose-efficient and robust simultaneous evaluation of pulmonary morphology, ventilation, vasculature, and parenchymal perfusion in a one-stop-shop procedure.

Abstract No. 923

Delineating the immune response of lung CD4+ and CD8+ T-cells to viral PAMPs using live microscopy

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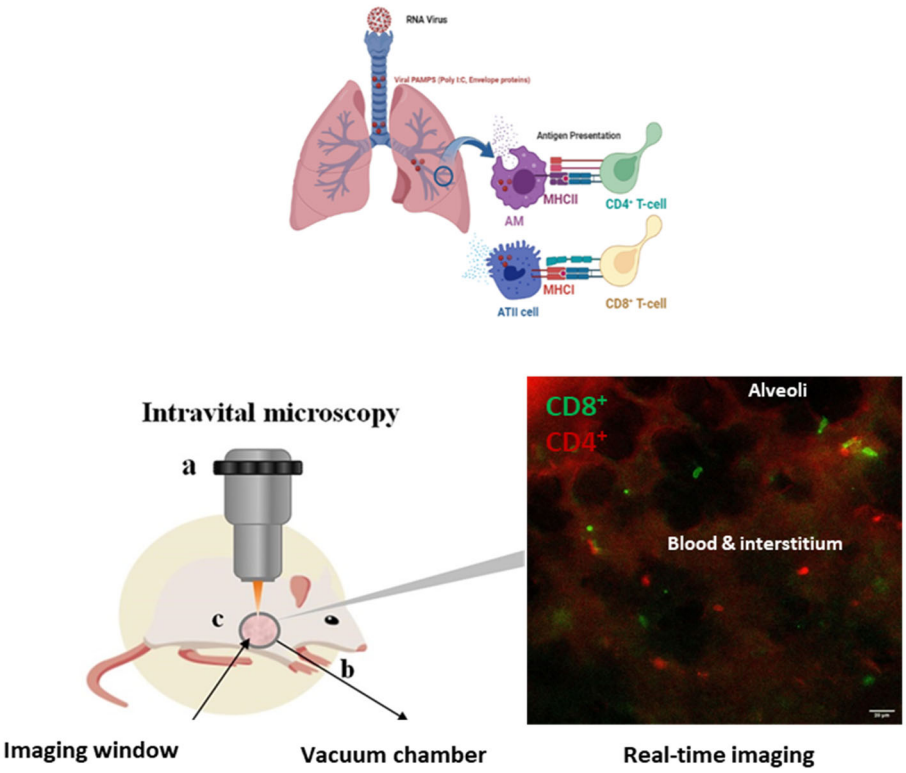
Aims & Methods: Delving deep into the mechanism of pathogenesis of RNA viruses infecting our lungs, like SARS-CoV-2, is a crucial step for the development of successful therapy and vaccination approaches. Lung intra-vital microscopy (IVM) using fluorescent antibodies or T-cell reporter mice is used for real-time spatial and temporal tracking of $CD4^+$ and $CD8^+$ T-cells in distal live lung, after intra-tracheal instillation with viral PAMPs, namely dsRNA viral mimetic Poly (I:C) and SARS-Cov2 Envelope (E) protein.

Results: Live microscopy of a coculture of ATII cells (MLE-12) and alveolar macrophages (MH-S) shows uptake of fluorescein-Poly (I:C) mainly by macrophages, which together with ATII cells contribute to the immune response. IVM shows the $CD4^+$ and $CD8^+$ T-cells, actively patrolling the pulmonary microcirculation in vivo. Immunophenotyping of CD4-Cre X mT/mG reporter mice via FACS shows GFP expression in both $CD4^+$ and $CD8^+$ T-cells, rendering this mouse a pan T-cell reporter. Intra-tracheal instillation of CD4-Cre mice with Poly (I:C) followed by IVM shows significant increase in the number of T-cells recruited to the alveolar region. Intra-tracheal instillation of C57BL/6J wild type mice with either Poly (I:C) or SARS-Cov2 (E) protein, followed by IV injection of fluorescently labelled $CD4^+$ and $CD8^+$ antibodies, shows significant changes in mean speed and net displacement of T-cells.

Conclusion: Monitoring the dynamics of lung immune cells can help unveil targets for therapy.



IVM is a valuable tool to study the immune response of T-cells to viral PAMPs in mouse lungs

Abstract No. 924

Inter- and Intra-Patient Correlations of T_1(TE) with Quantitative Perfusion in Patients with COPD

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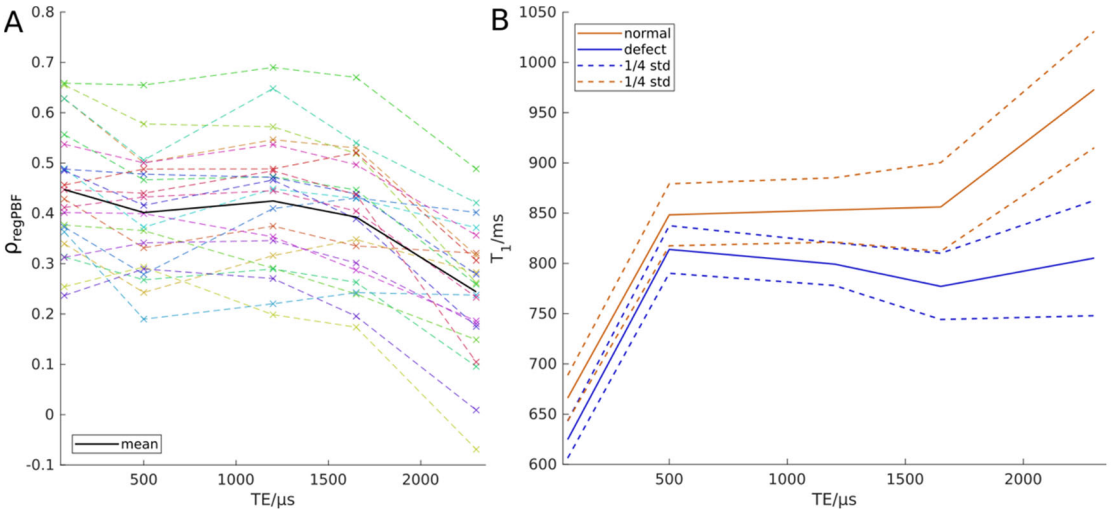
Objective: TE-dependent observed T_1 (T_1(TE)) mapping has potential for functional lung imaging. Inter-patient correlations between T_1(TE) and visual scores based on dynamic contrast enhanced (DCE) perfusion MR images have been shown. Here, we examined quantitative perfusion measures between patients (inter-patient) for entire lung slices and locally in patients (intra-patient). **Methods:** 26 patients with COPD (aged 68±7 years) were examined using an MRI protocol including 3D DCE MRI and T_1(TE) mapping using an inversion recovery multi-echo 2D ultra-short TE (UTE) sequence at 5 different TE (70µs to 2300µs) in 1-2 slices per patient. T_1 maps were calculated at each TE from the 2D UTE images. Lungs were segmented automatically and pulmonary blood flow (PBF) maps as well as defect classification maps used for defect fractions (QDP) were calculated from DCE images. These were then registered onto 2D UTE images using ANTs and Spearman's correlations (ρ) were calculated. **Results:** Inter-Patient correlations of up to $\rho_{IPBF,2}$=0.64 and $\rho_{IQDP,2}$=-0.76 and were found. Median T_1 was shorter in defect areas on average. Local ρ with PBF were lower than inter-patient ρ and weaker at TE_2 than at TE_1 and TE_3 on average. **Conclusion:** Inter-patient ρ were similar to previously shown ρ of T_1 with scores and strongest at TE_2. Local ρ were often much weaker, most likely due to lungs being in an overall severly damaged state. The lower ρ at TE_2 imply the TE-dependence of T_1 differs in defect and healthy areas.



A: Spearman's correlation coefficients for the correlation of T1(TE) with PBF. Each patient is shown with the same colour in both graphs. B: Mean of individual median T1 over all patients at each TE, according to defect classification.

Abstract No. 925

MDCT-based longitudinal automated airway and air trapping analysis in school-age children with mild cystic fibrosis lung disease

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Objectives: Quantitative computed tomography (QCT) offers some promising markers to quantify cystic fibrosis (CF)-lung disease. Air trapping may precede irreversible bronchiectasis; therefore, the temporal interdependencies of functional and structural lung disease need to be further investigated. We aim to quantify airway dimensions and air trapping on chest CT of school-age children with mild CF-lung disease over two years.

Methods and Materials: Fully-automatic software analyzed 144 serial spirometer-controlled chest CT scans of 36 children (median 12.1 (10.2–13.8) years) with mild CF-lung disease (median ppFEV1 98.5 (90.8–103.3) %) at baseline, 3, 12 and 24 months. Airway wall percentage ($WP_{5–10}$), bronchiectasis index (BEI), as well as severe air trapping (A3) were calculated for the total lung and separately for all lobes. Mixed linear models were calculated, considering the lobar distribution of $WP_{5–10}$, BEI and A3 cross-sectionally and longitudinally.

Results: $WP_{5–10}$ remained stable (P=0.248), and BEI changed from 0.41 (0.28–0.7) to 0.54 (0.36–0.88) (P=0.156) and A3 from 2.26% to 4.35% (P=0.086) showing variability over two years. ppFEV1 was also stable (P=0.276). A robust mixed linear model showed a cross-sectional, regional association between $WP_{5–10}$ and A3 at each timepoint (P<0.001). Further, BEI showed short-term longitudinal interdependencies with air trapping (P=0.003).

Conclusion: We could demonstrate cross-sectional, regional interdependencies between wall thickening and air trapping. Further, we show short-term regional interdependencies between air trapping and an increase in bronchiectasis. The data indicate that regional air trapping may precede the development of bronchiectasis.

Abstract No. 926

Pulmonary Artery Pulse Wave Velocity from Phase-Resolved Functional Lung MRI

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Introduction:
Pulse wave velocity (PWV) within the pulmonary arteries (PA) is an indicator for vascular stiffness, which is linked to mortality in patients with pulmonary hypertension (PH). The assessment via right heart catheterization is invasive, methods using phase-contrast (PC) MRI are restricted to measurements in one or two locations close to the heart [1]. Therefore, PA PWV calculation by phase-resolved functional lung (PREFUL) MRI is introduced.

Methods:
Coronal MRI slices were acquired at trachea bifurcation for 60 patients with suspected PH. PREFUL-MRI derived flow-related enhancement cycles [2] within each lung vessel voxel were compared to the PA by cross-correlation to identify the signals' time delay. Time delay and distance of the respective voxels were used to calculate PWV, the median value of all used voxels per patient was reported. PWV was compared to PC MRI using PA relative area change (RAC) and PWV derived from flow-area-loops (PWV-QA) using Spearman's correlation.

Results:
Median PREFUL PWV was 2,94 m/s (IQR: 1,90 m/s), median PWV-QA was 3,37 m/s (IQR: 2,16 m/s). PREFUL PWV in suspected PH patients was significantly correlated to RAC ($r = -0.42$, $p<0.001$) as well as to PWV-QA ($r = 0.39$, $p < 0.01$).

Discussion:
PA PWV calculation by PREFUL MRI is feasible and significantly correlated to arterial stiffness markers derived from PC MRI. The measurement is comprised of data points in multiple distal locations, which may account for differences to PWV measured by PC.

References:
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Abstract No. 927

Comparison of true non-contrast and virtual non- contrast images with Photon-Counting CT: evaluation of ex vivo perfused porcine lungs in an organ care system

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Objectives

To evaluate the performance of virtual non-contrast images (VNC) compared to true non-contrast images (TNC) images for the assessment of lung parenchymal attenuation

Methods

Four porcine lungs were ventilated and perfused ex vivo using the Organ Care System (OCS). Three scans were acquired, one without and two with different contrast concentrations using Photon Counting CT with mean vessel contrast of 30 HU (non-enhanced) and 150 / 300 HU in the contrast enhanced scans. Spectral post-processing was used to generate VNCs for the two contrast-enhanced scans. Mean values, SD, and percent volume of four density areas: Low attenuation (LAA, -1024 to -951 HU), normal attenuation (NAA, -950 to -601 HU), high attenuation (HAA, -600 to -250 HU), and very high attenuation (VHAA, > -250 HU) were calculated for TNC and VNCs.

Results

the mean attenuation difference between TNC and VNC attenuation was 6±1.4 HU, 5±0.4, -3±03, -7±5.9 HU for LAA, NAA, HAA, and VHAA respectively. Thus, TLC and VNC attenuation were equivalent in all tissues using a threshold of <10 HU difference. No systemic deviation was observed for the two iodine concentrations used in the enhanced scans. The mean lung volume difference between TNC and VNC was -2 ml, 58 ml, -24 ml, and -10 ml for LAA, NAA, HAA, and VHAA respectively, representing < 2% of total lung volume.

Conclusion

VNC is suitable for quantification of lung parenchymal attenuation as a substitute for TNC in routine clinical practice, resulting in a significant reduction in radiation exposure.

Abstract No. 928

Longitudinal MRI Detects Onset and Progression of Chronic Rhinosinusitis from Infancy to School-age in Cystic Fibrosis

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Background:
Chronic rhinosinusitis (CRS) contributes to morbidity in patients with cystic fibrosis (CF). Magnetic resonance imaging (MRI) was recently established to detect pathognomonic abnormalities of the paranasal sinuses from infancy. To date, longitudinal data on its onset and progression from infancy to school-age in patients with CF is lacking.
Methods:
64 children with CF (mean age at baseline 1.1±1.6 y, range 0-5 y) underwent a mean of 6±2 (range 3-11) consecutive annual MRI examinations of the paranasal sinuses. CRS was assessed using the previously evaluated CRS-MRI score.
Measurements and Main Results:
All maxillary and ethmoid sinuses were present from the first year of life, while sphenoid sinuses were developed in all patients from the age of 2 years and pneumatization of frontal sinuses started from the age of 3 years. The size of pneumatized sinuses increased according to age (r=0.475-0.798, *P*<0.001). In infancy, 76% of developed paranasal sinuses showed opacification, which increased towards school-age to 94% of paranasal sinuses (*P*<0.001). While in infants mucosal swelling was the dominant abnormality (58-97%), dominance of mucopyoceles increased with age and were most dominant in school-age children (in 48-66% of maxillary and 43-71% of frontal sinuses). The sinus subscores and CRS-MRI sum score were associated with age (r=0.148, *P*=0.02).
Conclusions:
MRI detects increasing prevalence and severity of paranasal sinus abnormalities from infant to school-age children with CF. Our data support the role of paranasal sinus MRI as a sensitive non-invasive modality for surveilling CRS in children with CF.

Abstract No. 929

Long-Term Effects of Lumacaftor/Ivacaftor on Chronic Rhinosinusitis in Children with Cystic Fibrosis Detected with Magnetic Resonance Imaging

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Rationale: Previous studies using magnetic resonance imaging (MRI) demonstrated early onset and progression of chronic rhinosinusitis (CRS) from infancy to school age in patients with cystic fibrosis (CF), and mid-term improvements of CRS in preschool and school-age children with CF treated with lumacaftor/ivacaftor for at least two months. However, long-term data on treatment effects on CRS in children with CF are lacking.
Methods: 39 children with CF homozygous for F508del (mean age at baseline MRI 5.9±3.0y, range 1-12y) underwent MRI before (MRI1) and about seven months after starting lumacaftor/ivacaftor and then annually (median 3 follow-up MRI, range 1-4) (MRI2-4). MRI were evaluated using the previously evaluated CRS-MRI score.
Results: The CRS-MRI sum score at baseline was similar in children starting lumacaftor/ivacaftor in school age and in children starting therapy at preschool age (34.6±5.2 vs.32.9±7.8, p=0.847). Mucopyoceles were the dominant abnormality in both, especially in maxillary sinus (65% and 55%, respectively). In children starting therapy in school age the CRS-MRI sum score decreased longitudinally from MRI1 to MRI2 (-2.1±3.5, p<0.05), MRI3 (-3.0±3.7, p<0.01) and MRI4 (-3.6±4.7, p<0.01), mainly due to a decrease in the mucopyoceles subscore. In children starting lumacaftor/ivacaftor in preschool age, the CRS-MRI sum score remained stable under therapy (0.6±3.3, p=0.520; 2.4±7.6, p=0.994; 2.1±10.5, p>0.999 and -0.5±0.5, p=0.740; respectively).
Conclusions: Longitudinal paranasal sinus MRI shows improvements in CRS severity in children with CF starting lumacaftor/ivacaftor therapy at school age. Further, MRI detects a prevention of an increase in CRS severity in children with CF starting lumacaftor/ivacaftor therapy at preschool age.

Abstract No. 930

3-D Registration for Lung Imaging in COPD: Towards Multi-modality Fusion and Analysis

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Objectives: Computed tomography (CT) and magnetic resonance imaging (MRI) are supplementary imaging techniques in assessment of chronic obstructive lung disease (COPD). Quantitative indices of emphysema, small airways disease(SAD), and perfusion were developed separately, and cross-correlation on patient level were proven. A voxel-wise correlation could provide further insight into contributions of emphysema and SAD on CT to perfusion abnormalities on MRI. We sought to develop a 3D registration pipeline in order to spatially align CT and MRI.
Methods: Patients with COPD underwent same-day non-enhanced low-dose CT and morpho-functional MRI incl. 4D perfusion MRI as part of the nationwide multicenter COSYCONET imaging substudy. In order to compensate intrinsically lower in-plane resolution in MRI, we interpolated coronal T1-weighted MRI to the resolution of CT through zero-filling in frequency domain. Lung segmentations for CT and MRI were generated separately by in-house software YACTA and by an established pipeline in Matlab. Afterwards, an iterative deformable registration was applied using Advanced Normalization Tools.
Results: We evaluated the pipeline on 20 patients with GOLD stage 0-4, Figure 1 shows pulmonary structures align well in the co-registered coronal CT and MR.
Conclusion: We propose a 3D registration pipeline to align CT and T1-weighted MRI. In the next step, the method will be integrated for the registration of additional MRI sequences incl. 4D Perfusion.

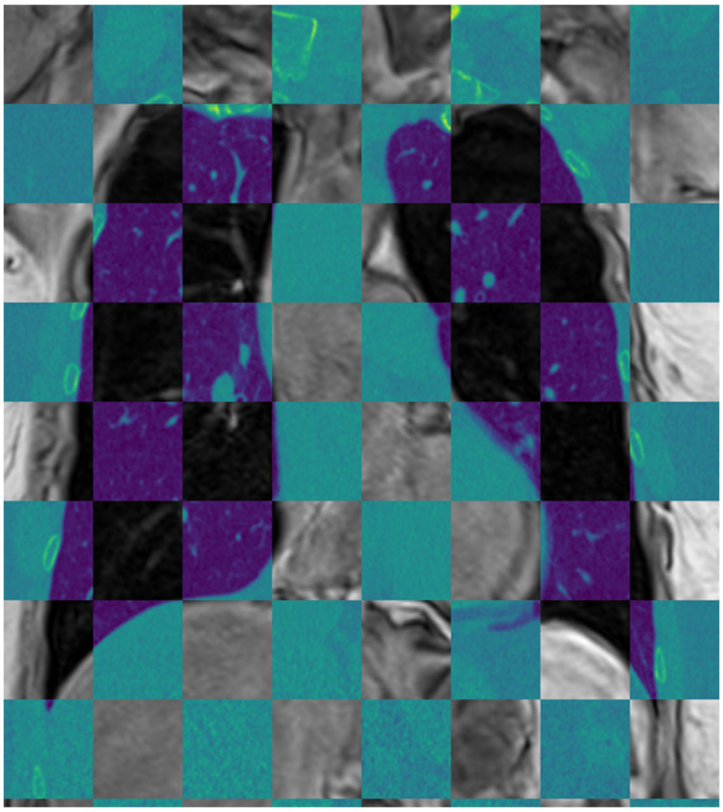

Figure. 1: Checkerboard comparison of an representative slice for co- registered CT and MRI, where colored patches represent CT intensity and gray scale patches represent MRI.

Abstract No. 931

Spatial zonation of ECM and cellular niches revealed by 3D volumetric imaging of cleared human lung tissues in health and disease

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Spatially-resolved characterization of human lungs at cellular resolution in large volumes is greatly limited by current *in vivo* computed tomography (CT) and *ex vivo* 2D HE histopathology. Traditional microscopic validation of biological findings and disease hallmarks generated from multi-omics technologies in clinical lung research often relies on thin 2D slices (3-8 µm), leaving the 3D anatomical connections and intricate molecular and cellular networks in health and disease understudied.

This study aims to develop a volumetric imaging technology for unraveling the complexity and interconnectivity of human lungs. We employed 3D light sheet fluorescence microscopy on optically-cleared lungs, ranging from healthy to end-stage diseases like COPD and IPF. We optimized whole-mount immunostaining methods to allow for deep tissue penetration of antibodies, heme removal, extracellular matrix loosening, and tissue autofluorescence reduction. We successfully visualized 3D tissue geometries and disease-specific patterns at the cellular level. 2D histological examination of optically-cleared human lungs revealed well-preserved lung tissue integrity and structural organization. We then validated the spatial zonation of a specific ECM protein from terminal to respiratory bronchioles in healthy lungs, which we recently identified using spatially-resolved mass spectrometry-based proteomics. We also characterized the 3D spatial organization of pulmonary and systemic vascular networks during lung fibrogenesis. Going forward we plan to systematically couple laser capture microdissection based spatial proteomics with 3D volumetric imaging of optically-cleared human lungs to further explore the spatial zonation of ECM and cellular niches in health and disease.

Abstract No. 932

Unveiling the complexities of pulmonary delivery: multimodal imaging and AI reveal temporally- and spatially-resolved nanoparticle dosimetry and macrophage trafficking in the lung

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Pulmonary delivery is vital for local therapy and understanding health effects of inhaled materials. Holistic view of pulmonary delivery and biokinetics of nanoparticles (NPs), along with the role of innate immunity in regulating the long-term fate of NPs in the lung, remains unclear. We employed advanced multimodal imaging and deep learning pipelines to reveal the complex (aerosol) deposition profiles and fate of biopersistent NPs in murine lungs at the cellular level.

Spatially-resolved delivery characteristics in entire airways and acini were revealed by tissue-clearing light-sheet fluorescence microscopy for six common routes of pulmonary delivery, namely intranasal and oropharyngeal aspiration, intubated instillation, microsprayer application, ventilator-assisted and nose-only aerosol inhalation. AI based whole-lung 3D segmentation of the entire airway was performed using nnU-Net to accurately determine bronchial (B) versus alveolar (A) NP dosimetry, revealing B/A ratios between 0.08 and 0.49 depending on the delivery route. The good correlation between B/A ratios and clinically used central (C) to peripheral (P) ratios provides direct proof for the widely used hypothesis that 2D C/P ratio is a valid indicator of 3D B/A ratios. Longitudinal 3D imaging showed similar intra-acinar NP kinetics independent of delivery route. In contrast to the conventional notion of passive diffusion, this study found that acinar NP relocation is mediated by phagocytosis and patrolling of tissue resident macrophages (TRMs), as evidenced by *ex vivo* and *in vivo* imaging and other analytical approaches. These findings provide a comprehensive understanding of NP-lung delivery and biokinetic features, as well as the role of TRM immunity in the fate of biopersistent NPs.

Contribution of a Disease-spanning Working Group: Lung Environment Interaction

Abstract No. 933

Optimizing vascular casting of adult and early postnatal mouse lungs for micro-CT imaging in preclinical studies

Zhe Zhou-Suckow[1], Qin Li[2], Willi Wagner[2], Jonas Labode[3,4], Jolanthe Schatterny[1], Martin Finke[2], Julia Schmidt[3,4], Mark Wielpütz[2], Hans-Ulrich Kauczor[2], Christian Mühlfeld[3,4], Rory E. Morty[1]

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Alterations to the pulmonary vasculature exist in many lung diseases. Vascular casting technique produces a replica of vascular beds of normal and pathological tissue. Little information is available that described the perfusion of the pulmonary vascular network in mice for radiological studies. We aimed 1) to optimize vascular casting of the pulmonary vascular tree in mice of different ages; 2) to establish an image analysis workflow for quantifying the structure of the pulmonary vascular tree; 3) to evaluate the pulmonary vasculature changes in the *Scnn1b*-Tg mouse model of cystic fibrosis-like lung disease. Mice of postnatal ages of 3, 5 and 7 days, and 2, 3 and 5 weeks were anesthetized and heparinized, a PE-10 catheter was inserted into the pulmonary artery, and the vasculature was flushed of blood via a syringe pump. The lungs were inflated via the trachea at a standard pressure. Microfil compound was then infused into the vascular bed. After filling and curing, the lung vasculature was imaged at 9 µm resolution on a micro-CT scanner. For branching analysis, the arterial tree was divided into its individual segments classified based on the Strahler order. The fractional vessel volume and vascular surface density of the peripheral vascular bed of pulmonary arteries of 6-week-old *Scnn1b*-Tg mice were significantly lower than WT controls, indicating the reduction of vascular bed in the diseased mice. We improved the pulmonary artery injection in catheter placement/stabilization, set up a guideline for the flow rate/volume, and established a workflow for quantifying the pulmonary vascular tree structure. Our data provide important groundwork for the radiological imaging of the pulmonary vasculature, for studies on bronchopulmonary dysplasia and pulmonary hypertension.

Abstract No. 934

Non-invasive estimation of mean pulmonary arterial pressure using Deep Learning and PREFUL-MRI

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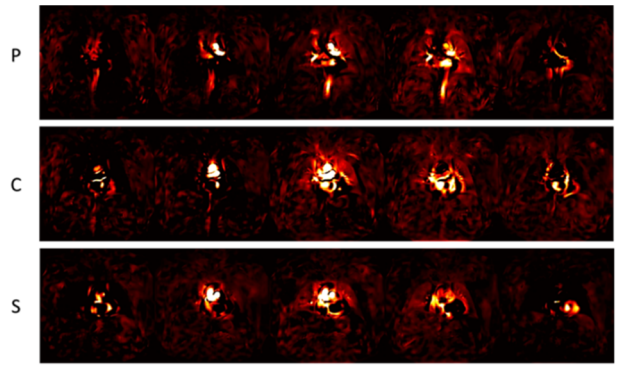
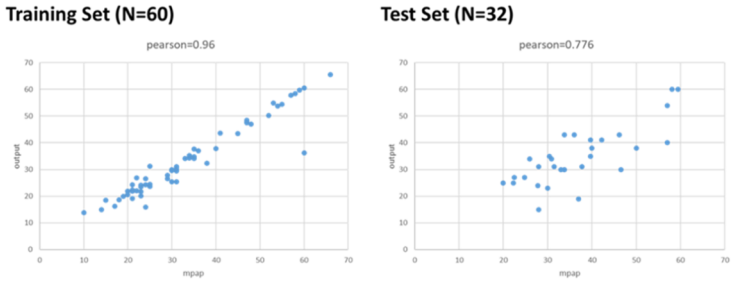
Introduction: Mean pulmonary arterial pressure (mPAP) is a biomarker of pulmonary hypertension (PHT), which is currently determined by right heart catheterization (RHC) as the clinical standard. [1] Phase-resolved functional lung MRI (PREFUL) [2] is a non-invasive imaging technique which can calculate the pulse wave propagation in pulmonary vasculature during a virtual cardiac cycle of 15 phases. We hypothesize that deep learning tools may be able to estimate mPAP from dynamic pulse wave propagation images derived by PREFUL. **Method:** 92 (f:48, PHT: 57) subjects underwent PREFUL and RHC. Per patient, pulse wave propagation images of coronal, central slice as well as the next ventral and dorsal location in the thorax were combined as input data (Figure 1a). mPAP given by RHC was defined as target output data. Finally, a combination of three Denset-201 with one regression network was trained to realize the aforementioned mapping with 60 subjects and tested with 32 remaining cases. The correlation of the network's final output with the mPAP was measured using Pearson's Correlation. **Results:** As shown in Figure 1b, the final output of our non-invasive method showed a significant ($p<0.05$) correlation with mPAP measured by RHC for both training and unseen test data. **Conclusions:** Non-invasive measurement of mPAP using PREFUL-MRI and deep learning appears feasible and needs to be further explored.



a) Sample of pulse wave propagation images for centered slice (c) and its predecessor (p) and successor (s)



b) Correlation between network output and mPAP from RHC

Figure 1: Sample of input data and resulting correlation with mPAP

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