DZL
Deutsches Zentrum für
Lungenforschung

DZG DEUTSCHE ZENTREN
DER GESUNDHEITSFORSCHUNG

German Center for Lung Research

ANNUAL REPORT

2013

DZL Highlights 2013

Translational Highlights

- Establishment of a pediatric asthma cohort
- International approval of Riociguat for the treatment of pulmonary arterial hypertension and chronic thromboembolic hypertension (first drug in this disease) with DZL scientists as leading investigators
- Development of an “organ care system” allowing prolonged extracorporeal lung perfusion for transplantation and novel treatment approaches
- Novel technology for assessment of airway inflammation by magnetic resonance imaging
- Molecular dissection of pathogen – host cell crosstalk driving severe influenza pneumonia
- Identification of a novel biomarker (micro-RNA 142-3p) to discriminate between good and poor prognosis in patients with adenocarcinoma

Strategic Highlights

- CAPNETZ joins DZL as associated partner
- Kickoff of German French Lung School
- DZL Annual Meeting in Bad Nauheim – attended by more than 300 participants
- DZL International Symposium in Munich – attended by close to 200 participants
- DZL founds Technology Transfer Consortium

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Foreword

Lung diseases rank second with respect to morbidity and mortality worldwide. The World Health Organization lists four lung diseases among the top ten causes of death on a global level, and every fifth death is caused by lung disease or the consequences thereof. The European Respiratory Society estimates the direct and indirect costs of lung disease are more than 100 billion € per year for Western Europe alone. For most respiratory diseases currently available treatments provide symptomatic relief but no cure. Moreover, the incidence and economic burden of lung disease are expected to increase over the next decades. For these reasons, it is more important than ever to develop new approaches for combatting lung disease, including options for disease prevention, diagnosis, and therapy. Supported by German Federal and State Governments, the DZL brings together leading scientists and clinicians throughout Germany in the field of pulmonary research, all united with the aim of developing innovative new therapies for patients with lung disease.

2013 was a busy and productive year for the DZL. DZL scientists and clinicians made significant progress on many research fronts from leading the way towards approval of Riociguat for the treatment of pulmonary arterial hypertension as well as chronic thromboembolic pulmonary hypertension, the first drug in this disease, to new paradigms for lung allocation in transplant patients. The DZL welcomed CAPNETZ as a new partner, celebrated the launch of the German-French Lung School, had more than three hundred participants at its 2013 Annual Internal Meeting in Bad Nauheim, and hosted its second International Symposium. We invite you here to learn more about these and other 2013 DZL highlights.

Professor Dr. Werner Seeger
Chairman and Speaker of the DZL

About the DZL

Founded in 2011, the German Center for Lung Research (Deutsches Zentrum für Lungenforschung, DZL) is one of six German Centers for Health Research (Deutsche Zentren der Gesundheitsforschung, DZG). The DZGs are the result of an initiative of the German Federal Ministry of Education and Research (Bundesministerium für Bildung und Forschung, BMBF). Through an innovative structure of partnerships between top universities with university hospitals and non-university research institutions throughout Germany, a key aim of the DZGs is the development of new therapeutic options for major public health issues.

In the DZL 200 principal investigators and their research groups have banded together to fight pulmonary disease through translational research. Basic science and disease- and patient-oriented research in the field of lung disease are coordinated through an integrated approach devoted to improving patient care as quickly and efficiently as possible. DZL scientists are located at 22 top research institutes throughout Germany, and their activities are managed by five coordinating centers: Airway Research Center North (ARCN), Biomedical Research in Endstage and Obstructive Lung Disease Hannover (BREATH), Comprehensive Pneumology Center Munich (CPC-M), Translational Lung Research Center Heidelberg (TLRC), and the Universities of Giessen and Marburg Lung Center (UGMLC). The DZL is supported by the BMBF and the states which house DZL partner institutions.

In order to achieve its scientific and clinical goals, the DZL is organized around the concept of Disease Areas. Each Disease Area team consists of clinicians and basic scientists working together to advance treatments and therapies for a specific indication. Working in concert with the DZL Executive Board, elected Disease Area leaders set goals and milestones for each Disease Area and monitor progress. Each Disease Area team is supported by a coordinator, and progress is reported to the DZL Executive Board no less than every six months. Not working in isolation, each Disease Area team has access to common infrastructure and resources. Furthermore, many investigators belong to more than one Disease Area team, allowing for cross-fertilization of ideas and findings across research areas.

Science – Translation in Focus

The DZL has the mission of using translational research to combat widespread lung diseases. Translational research is the process of transforming scientific discoveries arising from the laboratory into practical applications that directly impact human health and well-being. While some translational research programs focus exclusively on translation of existing basic science findings into clinical practice, the DZL believes that successful translational science can only be achieved via an iterative process including both clinical and basic research.

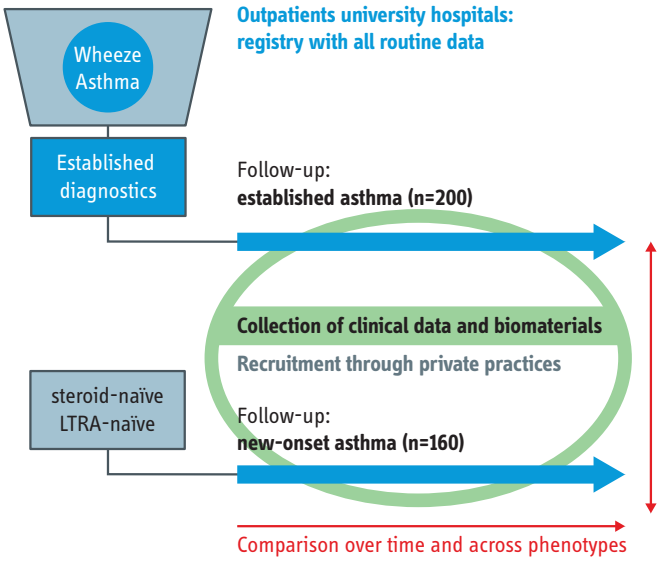
With the mission of using "translational research to combat widespread lung diseases," research efforts in the DZL are focused on eight disease areas: asthma and allergy, chronic obstructive pulmonary disease, cystic fibrosis, pneumonia and acute lung injury, diffuse parenchymal lung disease, pulmonary hypertension, endstage lung disease, and lung cancer. For each of the diseases studied by DZL scientists, the entire "bench-to-bedside" translational research chain is applied. Basic science findings inform design and implementation of clinical trials and patient care, and clinical needs drive the basic science questions tackled by DZL scientists. The close integration of basic scientists and clinicians is integral to the success of the DZL and is facilitated by regular meetings, symposia, and access to common infrastructure.

Asthma and Allergy

Disease Area Leaders	Prof. Dr. Heinz. Fehrenbach (ARCN) Prof. Dr. Erika von Mutius (CPC-M)
Participating DZL Partner Sites	ARCN, BREATH, CPC-M, TLRC, UGMLC
Number of Participating DZL Faculty	43

Asthma is the most prevalent chronic respiratory disease in childhood and is also very common in adults. Although the clinical manifestations of asthma in children and adults are rather uniform with wheezing, shortness of breath, and cough, population-based clinical and genetic studies suggest that asthma is not one disease, but many. Thus, a single "one-size-fits-all" treatment approach is unlikely to work to tackle this important health problem. In order to design personalized treatment approaches for asthma patients, there is urgent need to elucidate the mechanisms underlying the various types of asthma. The decoding of such mechanisms and their translation to the individual patient is the aim of the Disease Area Asthma and Allergy of the DZL.

In order to decode mechanisms that may underlie development of distinct wheeze and asthma phenotypes in childhood, the Disease Area follows two approaches.



(1) A clinical registry is being created, which includes all clinical data of all patients with wheeze and asthma seen in asthma clinics in three centers: ARCN, BREATH, CPC-M. (2) In order to perform deep phenotyping with the goal of identifying possible biomarkers for distinct wheeze and asthma phenotypes in childhood, a clinical cohort will be created. This cohort will allow for detailed characterization (deep phenotyping) of young wheezy children and asthmatic adolescents, respectively, (a) for children with established diagnoses ("established asthma", n=200, upper part) and (b) for children with new-onset disease, which are – due to their early disease stage – still naïve with regard to the use of medications that may impact immune responses, i.e. steroids and leukotriene receptor antagonists (n=160, lower part). Both children in the registry and the cohort will be followed-up yearly in addition to clinical routine visits until at least 2015 and possibly beyond.

For children with established diagnoses and those with new-onset disease, the collection of biomaterial, clinical data and clinical measurements such as lung function and markers of allergic airway disease continued throughout 2013 (green circle). These data may aid in identifying biomarkers for distinct wheeze and asthma phenotypes in childhood that can be discovered in the clinical setting and replicated in children with new-onset disease, for whom disease course can be followed more precisely but who will more often be seen in primary care setting as opposed to a clinical setting due to their premature disease state (red arrows).

LTRA: leukotriene receptor antagonist

Goals followed in 2013 – Asthma and Allergy

Goal 1 – German Collaborative Asthma Cohort

- Building an asthma and allergy patient registry, crossing the gap between pediatric and adult asthma
- Comprehensive clinical characterization of enrolled patients
- Collection of biomaterials for high throughput methods
- Integrating clinical and "omics" data by means of systems biology approaches

Goal 2 – Mechanisms Underlying the Development of Asthma Phenotypes

- Translational models of asthma phenotypes
  - Establishment of novel phenotype-specific murine models (incl. transgenic models) for mechanistic (e.g. the role of granulocytes, T and B cells in pathogenesis) and pre-clinical studies
  - Generation of Drosophila models for the functional characterization of novel candidate genes for asthma
- Cellular mechanisms
  - Identification of structural and functional properties of allergens that can lead to qualitatively different immune responses (dimer/oligomer formation; epitope mapping)
  - Characterization of the role of airway epithelium in the formation of distinct asthma phenotypes (epithelial signatures)
  - Identification of individual genes and pathways in tissues of the epithelial-mesenchymal trophic unit and nervous system with key features in the pathogenesis of asthma (remodeling, bronchoconstriction)
  - Analysis of the importance of the innate immune system in the pathogenesis of distinct asthma phenotypes
  - Identification of phenotype-specific components of the adaptive immune system (imprinted phenotypes, cell differentiation, role of specific cell subtypes, chipcytometry)
  - Identification of new biomarkers and molecular targets for asthma phenotypes
  - Establishment and application of a lipidomics platform
- Genetic, epigenetic, and microbiome analyses
  - Human genome and epigenome analyses
  - Analysis of epigenetic signatures (in particular chromatin modifications) in human BAL and blood samples from an asthma cohort
  - Establishment and use of systems biology platform

2013 Research Highlights – Asthma and Allergy

Research Highlight #1: Registry and Cohort

a. Methods development, implementation of strict quality control across centers, and recruitment

Methods development
Finalization of design, ethics and data safety
Finalization of 7 questionnaires, translation into electronic case report forms, database programming
Finalization of consent forms
Setup of recruitment within asthma clinics (registry; deep phenotyping of children with established diagnosis – cohort)
Setup of recruitment through private practices (deep phenotyping of steroid- and LTRA-naïve children with wheeze – cohort)
Setup of laboratory methods in 3 clinical centers (ARCN, BREATH, CPC-M) and 10 laboratories

Implementation of strict quality control
Finalization of 48 standard operating procedures for all clinical and lab modules
Site visits lung function (adherence to existing guidelines)
Standardization of lab methods by training visits across centers
Setup of laboratory site visits

Recruitment through end 2013 – Registry		Recruitment through end 2013 – Cohort (steroid-/LTRA-naïve children)	
Screened	n=806	Screened	n=91
Eligible	n=594	Eligible	n=73
Included	n=527 (88.7% of eligible)	Included	n=71 (97.3% of eligible)

b. Crossing the gap from pediatric to adult asthma, implementation of transition clinics

Implementation of pediatric study design for the study of adult asthmatics by ARCN in order to retrospectively assess clinical course during childhood
Ethics approval
Data safety
Adaptation of lab methods and standard operating procedures implemented for pediatric studies
Adaptation of questionnaires implemented for pediatric studies
First steps to establish transition clinics

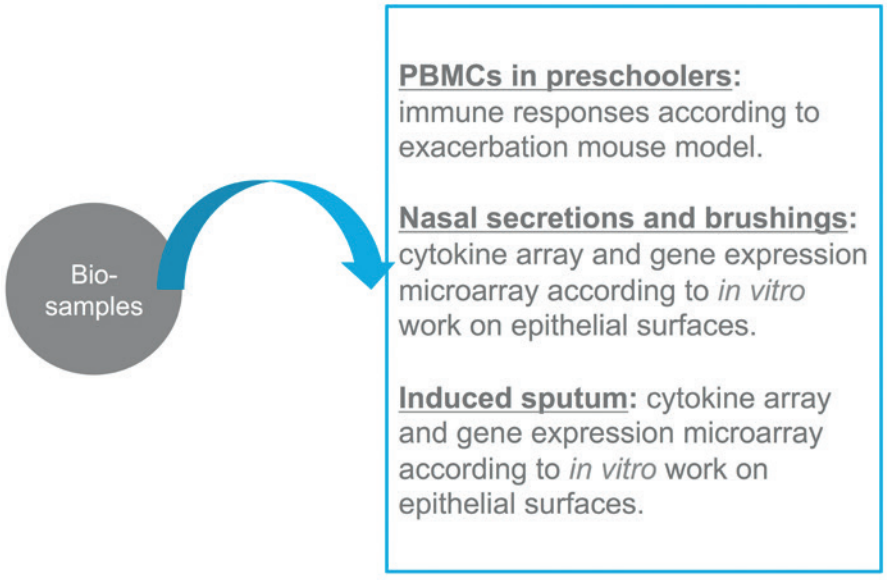


Figure 1. Cohort – Transfer to experimental studies

The analysis of primary cells from blood and from nasal epithelia collected by nasal brushings as well as of nasal secretions and of induced sputum serves are examples of biomaterial analysis in the Disease Area Asthma and Allergy. Closing the gap between human and mouse studies, PBMCs will be tested in line with data collected in mouse experiments. Nasal secretions and primary cells will be analyzed by cytokine arrays which are designed based on preliminary results from in vitro data from standardized cell lines. The same will be done with human biosamples collected by induction of sputum.

PBMCs: peripheral blood mononuclear cells

Biosample Analysis

The analysis of biosamples from the asthma cohort is a highly cooperative process, both within the DZL and beyond as described in figures 2a–c.

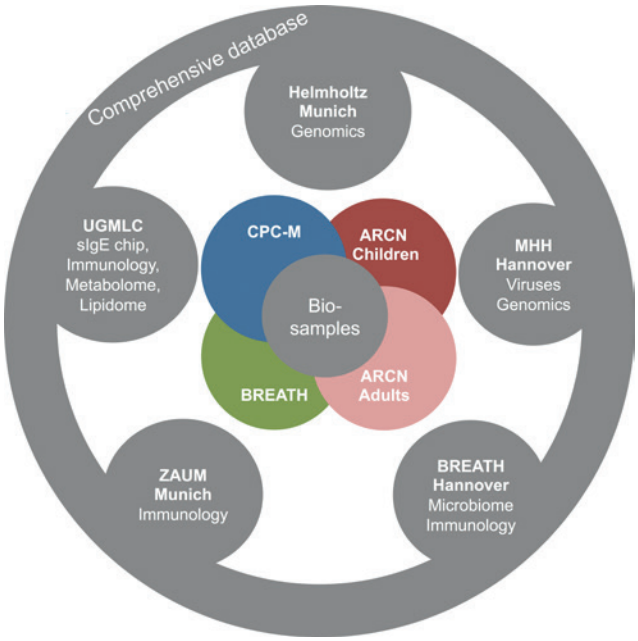


Figure 2a. Cooperation Within the Disease Area Asthma and Allergy

In order to enable deep phenotyping of children with wheeze and asthma, collection of biosamples in all clinical centers (ARCN-children, BREATH, CPC-M) including the collection of samples from adult asthmatics (ARCN-adults) and procedures for distribution of these biosamples to analyzing laboratories have been implemented. All results of these analyses are collected in a common comprehensive database.

Figure 2b. Cooperation Within the DZL

In order to compare both clinical data and biosamples from children with wheeze and asthma as well as with cystic fibrosis, for whom early lung disease may follow similar patterns, a cooperation between the Disease Area Asthma and Allergy and the Disease Area Cystic Fibrosis has been established. CF: cystic fibrosis

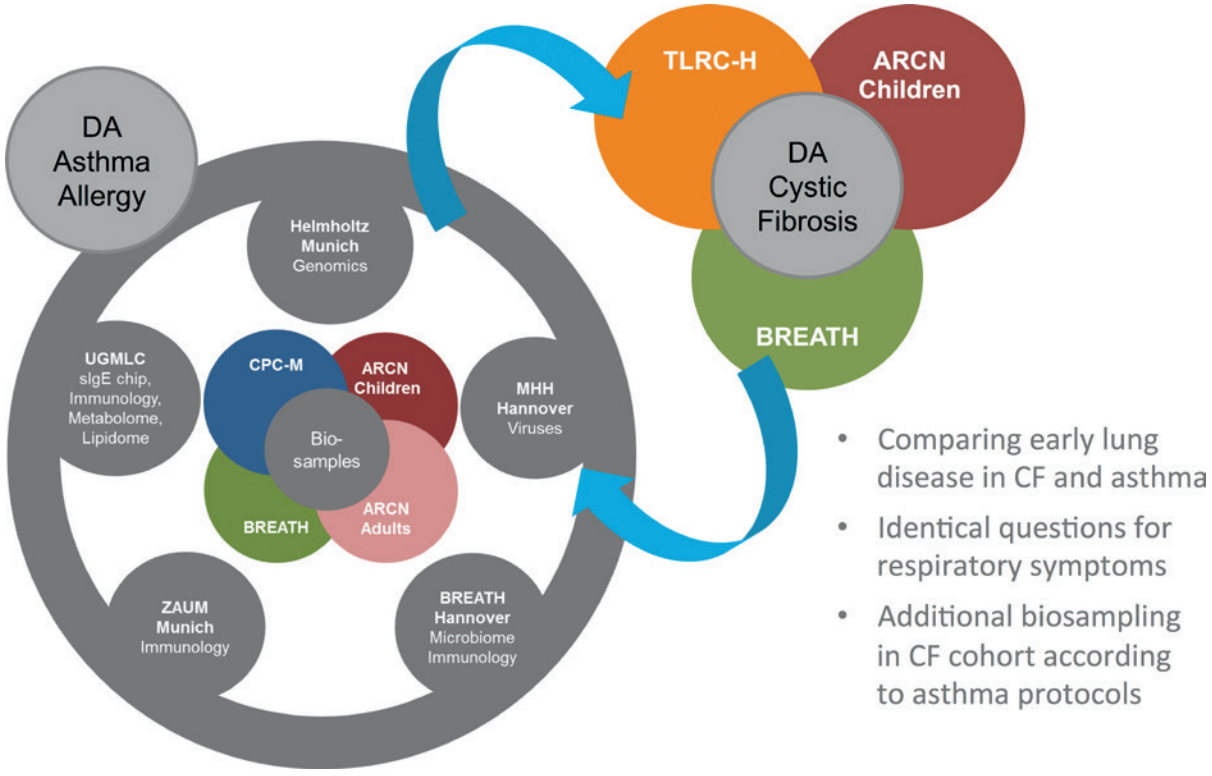
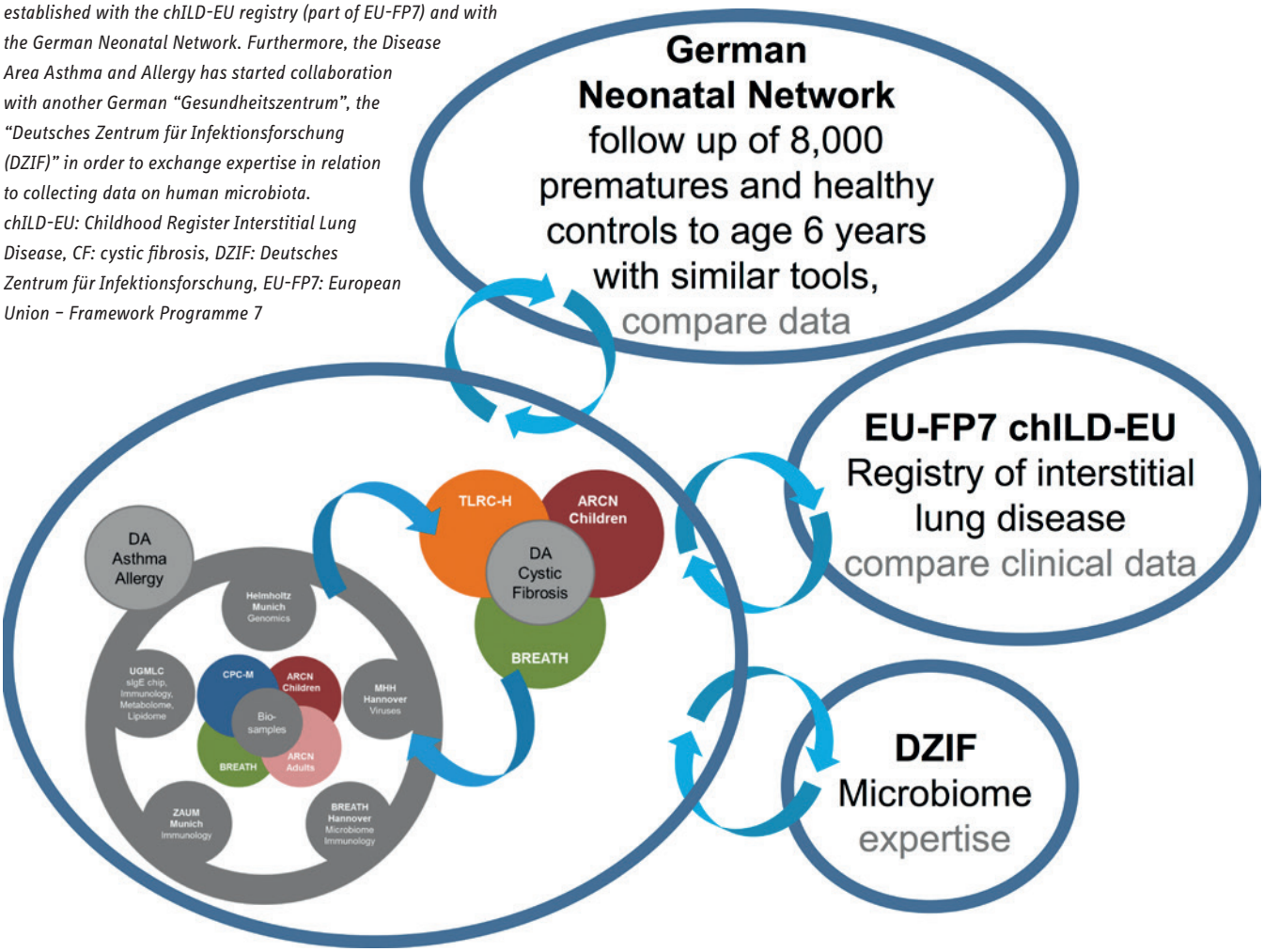


Figure 2c. Cooperation Within the DZL

In order to extend collection of clinical data to children with interstitial lung disease and children born prematurely, further cooperations have been established with the chILD-EU registry (part of EU-FP7) and with the German Neonatal Network. Furthermore, the Disease Area Asthma and Allergy has started collaboration with another German "Gesundheitszentrum", the "Deutsches Zentrum für Infektionsforschung (DZIF)" in order to exchange expertise in relation to collecting data on human microbiota. chILD-EU: Childhood Register Interstitial Lung Disease, CF: cystic fibrosis, DZIF: Deutsches Zentrum für Infektionsforschung, EU-FP7: European Union – Framework Programme 7



Basic scientists within the Disease Area Asthma/Allergy address three major questions with direct relevance for the translation of results from basic research into clinics: a) the identification of specific pathogenetic mechanisms that are central to the development of virus induced exacerbations, b) the analysis of nasal secretions and epithelial cells obtained by brushings, and c) the analysis of induced sputum as a "window" through which processes that are active in the lungs can be observed.

In 2013 a number of cooperative initiatives across several DZL sites addressed these three major topics resulting in the establishment of novel animal models and the investigation of new aspects of the disease in established models (see Research Highlight #2), the harmonization of methods (via the establishment of common SOPs), their validation or advancement, respectively as well as initial validation studies (see Research Highlight #3).

Research Highlight #2: Translational models of asthma phenotypes.

The DZL junior research group "Asthma Mouse Models" that was recently set up at the ARCN site established a mouse model reflecting the acute, virus-triggered exacerbation of already established allergic bronchial asthma. Local stimulation of TLR-3 mirroring the presence of double stranded, viral RNA was sufficient to aggravate allergic airway inflammation (including marked infiltration of neutrophils), goblet cell hyperplasia as well as airway hyperreactivity. In further experiments with Il-17 knock-out mice it was demonstrated that this acute exacerbation is strictly dependent on IL-17. These data were part of a PhD thesis, which was awarded the Deutsche Lungenstiftung prize for "Best Experimental Dissertation.". Currently, the awardee works on identifying the cellular source of IL-17.

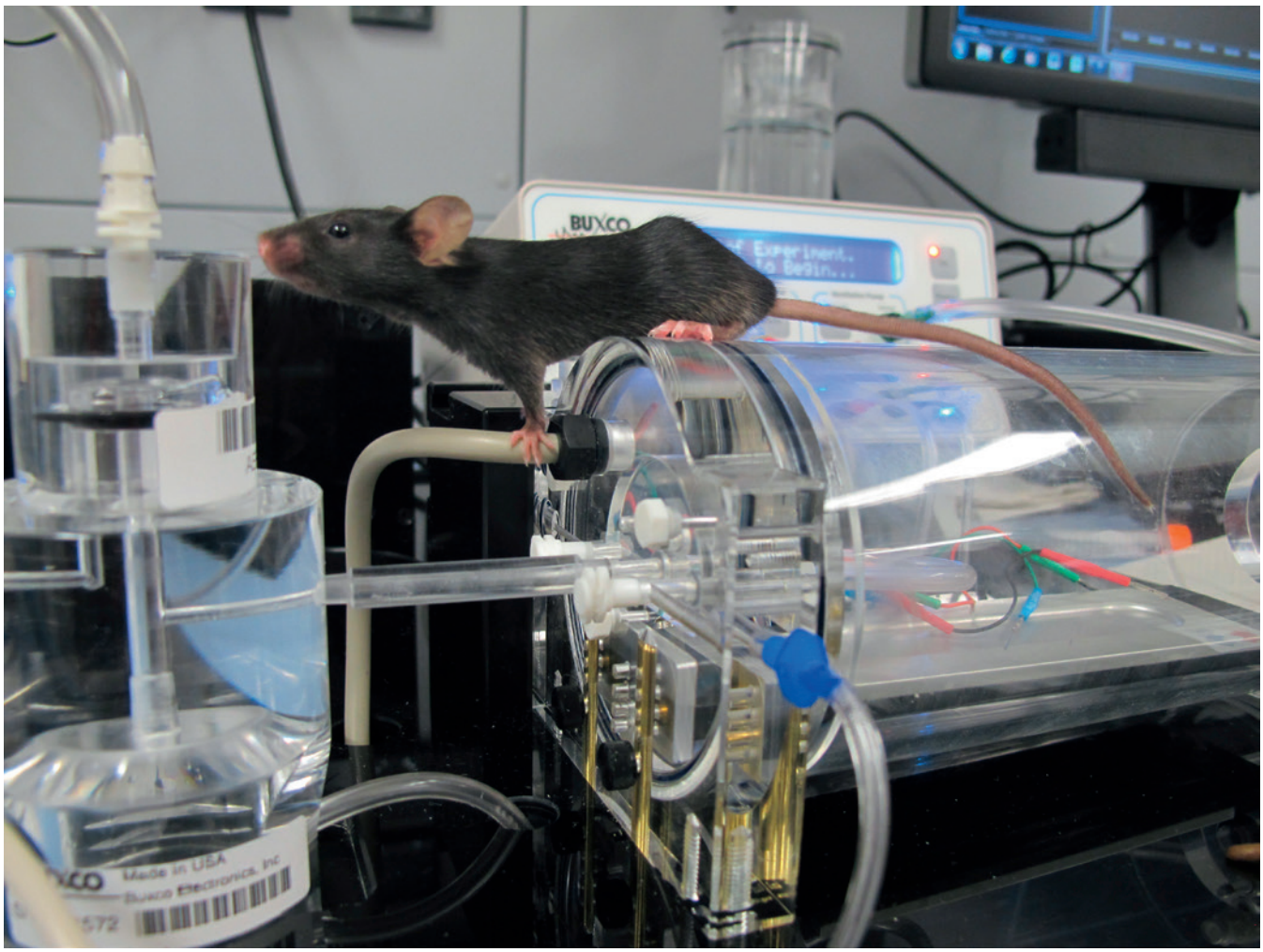


***Figure 3. C57BL/6 mouse is interested in the system used for measuring lung function**, i.e., determination of airway resistance and its alteration by methacholine (Figure reprinted with kind permission of Dr. M. Wegmann, FZB)*

In the lung, lymph vessels are important for the drainage of fluid and for the migration of immune cells and consequently are involved in many lung diseases. Until recently, the examination of the lymphatic system in mouse models of lung diseases was hampered by the lack of markers to unequivocally identify lymph vessels in the murine lung. As described in the paper by Kretschmer et al. we established antibodies to CD90/Thy-1 as markers for murine lung lymph vessels. They allow the visualization of lymphatic vessels in precision cut lung slices in healthy as well as in allergically inflamed lungs to better understand the migration routes of immune cells in the lung in steady state and during inflammation. Furthermore, the marker can also be used to label lymph vessels in 2-photon microscopy to directly visualize immune cell trafficking into lymph vessels. In current work, the method was refined to quantify the number of lymph vessels in the lung. By using this method, we could identify hypoxia as a strong inducer of lymphangiogenesis in the lung.

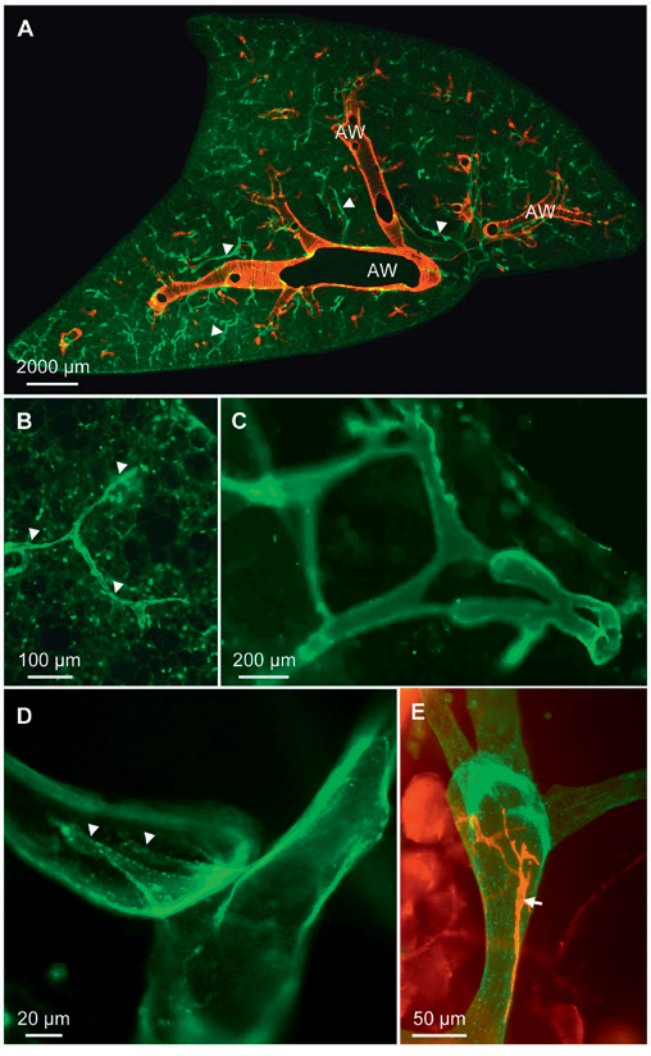


Figure 4. Immunohistochemistry of CD90/Thy-1 in murine precision cut lung slices. *A) The anti-CD90/Thy-1 antibody (green) stains the vascular system (arrowheads) in murine precision cut lung slices. Red staining shows immunoreactivity for α-smooth muscle actin. AW: airways. B) Initial CD90/Thy-1-immunoreactive capillaries (arrowheads) in the alveolar region. C) The CD90/Thy-1-immunoreactive vascular network is interconnected. D) A CD90/Thy-1-immunoreactive valve (arrowheads). E) α-smooth muscle actin-immunoreactive cells (red, arrow) were found on CD90/Thy-1-immuoreactive vessels only close to the hilum.*

(Figure from: Kretschmer T et al. Visualization of intrapulmonary lymph vessels in healthy and inflamed murine lung using CD90/Thy-1 as a marker. PLoS ONE 2013; 8(2): e55201)

Research Highlight #3: Identification of new biomarkers and molecular targets for asthma phenotypes

Induced sputum is an excellent source for the investigation of inflammatory processes in the airways by non-invasive means. In order to facilitate analysis of the transcriptome of the cells present in induced sputum, dithiothreitol (DTT) is used to chemically reduce disulfide-bonds of glycoproteins of mucus. The present study demonstrated that treatment with DTT significantly affects the results obtained by transcriptome analysis: a total of 1,384 genes were affected. On the basis of log-2-fold changes, the expression level of 917 genes was increased whereas in 467 genes the expression level was reduced. The methods validated along this line will be implemented into the analyses of transcriptomes of induced sputum within the cohort deep phenotyping studies.

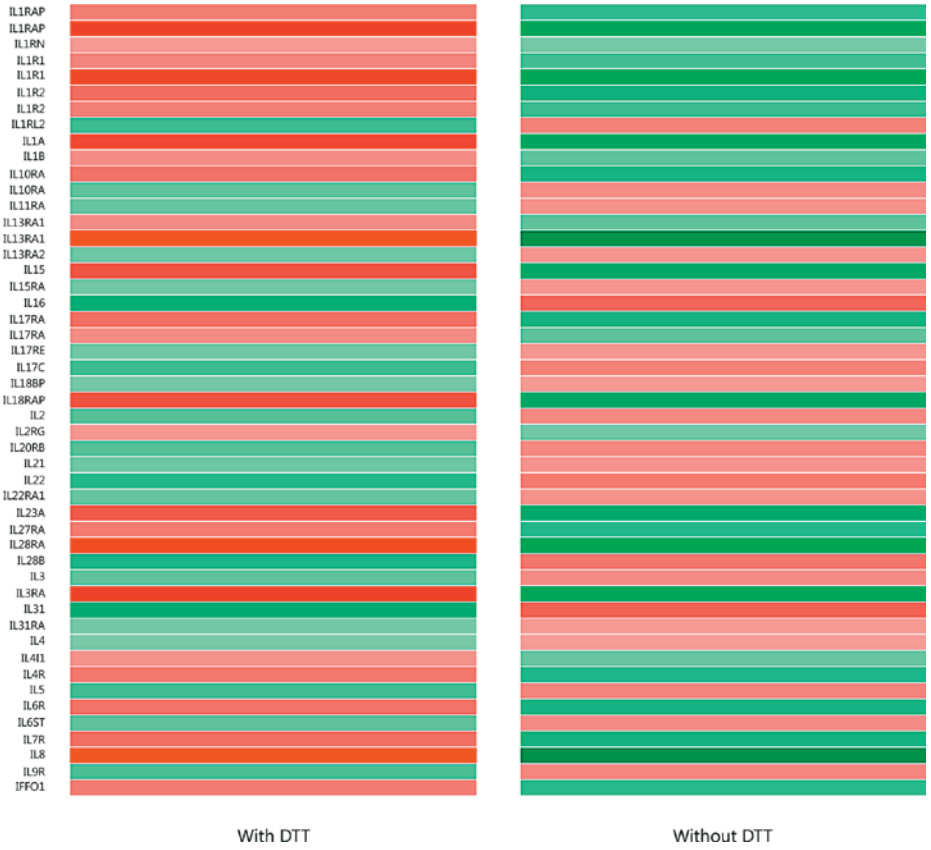


Figure 5. Heat map of transcriptome analysis of HOPE-preserved induced-sputum RNA processed with and without DTT. *Agilent (4 × 44K) human whole genome arrays were used. Analysis of the results was performed with a fold-change analysis using Gene-Spring 12 software. Red color intensity indicates higher expression, green color intensity indicates lower expression and white indicates a medium level of expression of a gene in the comparison of both groups. Gene description is shown on the left-hand side.*

(Figure from: Goldmann T et al. The effect of dithiothreitol on the transcriptome of induced sputum cells. Respiration 2013; 86:262–263. Copyright © 2013, Karger Publishers)

For functional phenotyping of human B cells (as planned during analysis of PBMC from the cohort's samples), an approach for high-content phenotyping of human B cell activation was developed and validated in a separate study. In combination with microarray analysis, high-content chipcytometry was used as a biomarker screening tool on sorted B cell populations to identify a set of 10 biomarkers for future deep phenotyping of B cell activation in patient samples.

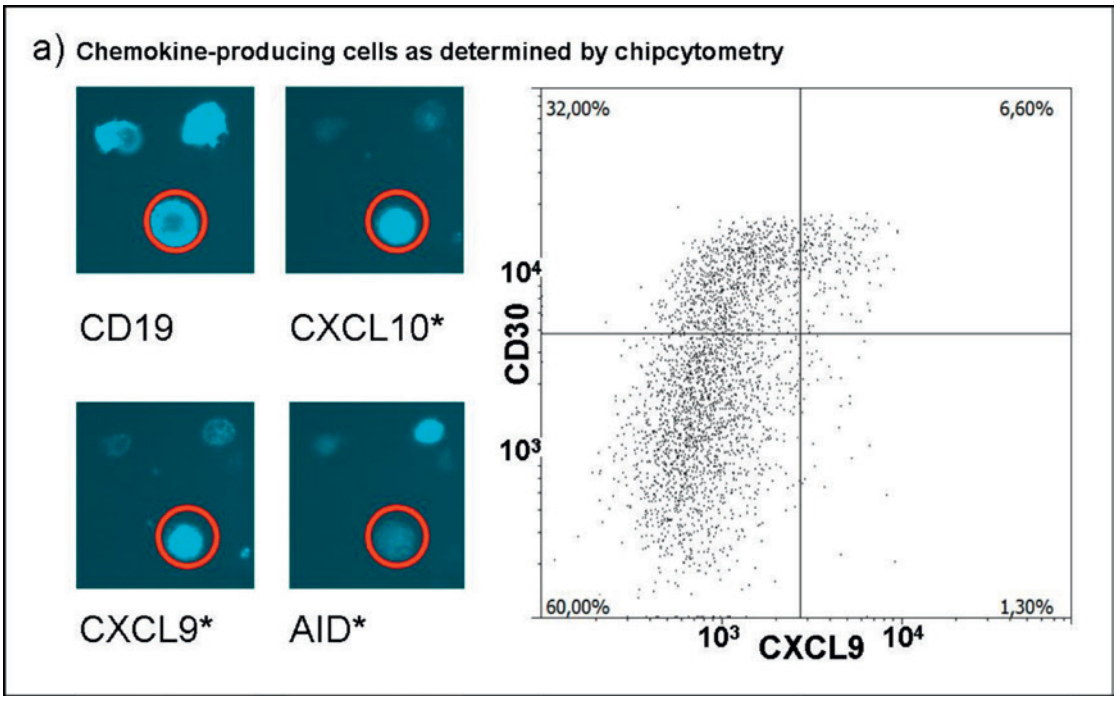


Figure 6. CD40L/IL-21-activated, non-class-switched B cells develop an extra-follicular CD30+ phenotype. *They were identified as the source of a novel CXCL9 and CXCL10 chemokine production. Image cytometry of intracellular CXCL9/ CXCL10 in CD30+ B cells. AID: activation-induced cytidine deaminase.*

(Reprinted from: Hennig C et al. High-content cytometry and transcriptomic biomarker profiling of human B-cell activation. J Allergy Clin Immunol 2014; 133:172-80, ©2014 with permission from Elsevier)

Number of papers published by DZL Faculty in 2013 – Disease Area Asthma and Allergy: 77

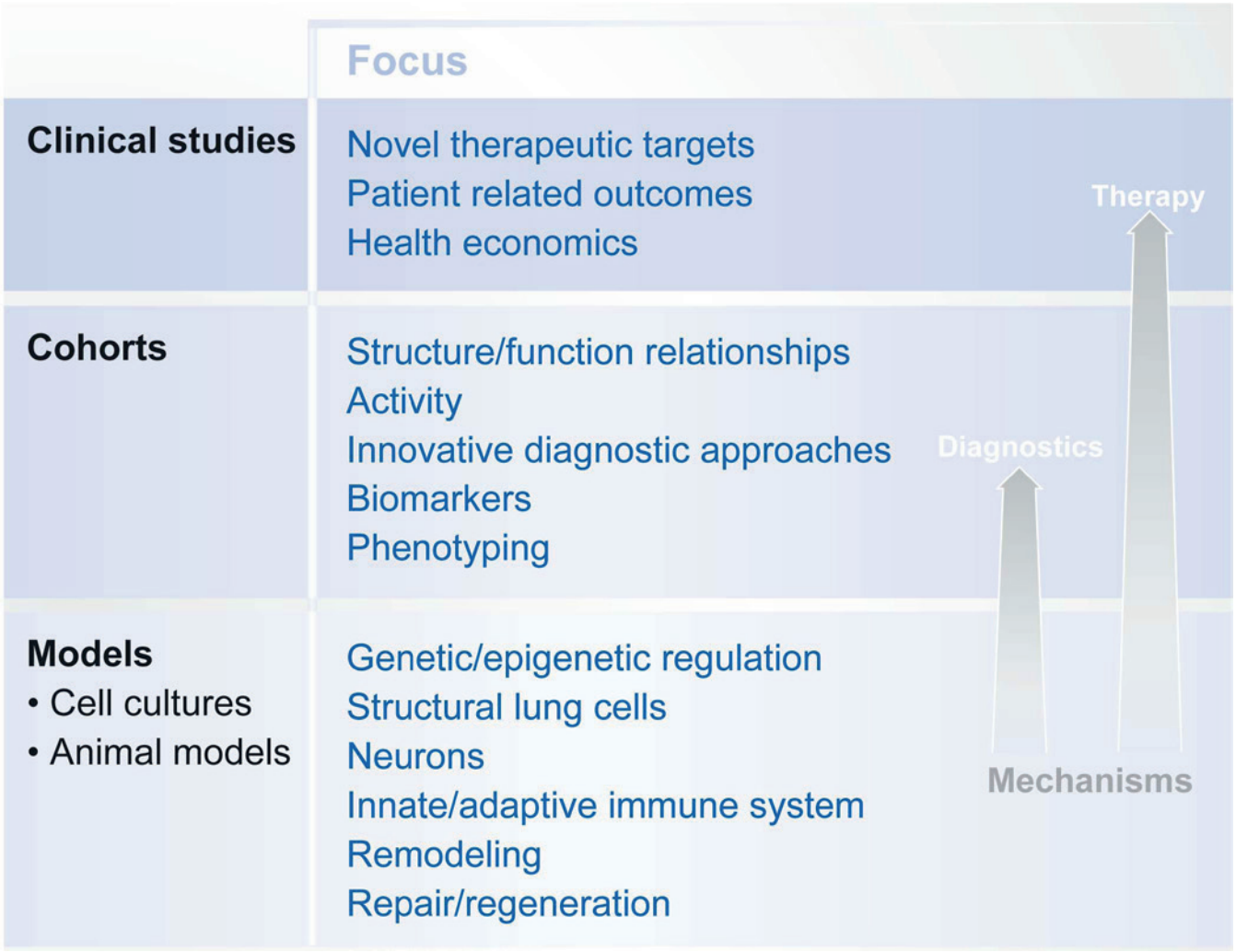
Highlighted Publications

1. Depner M, Fuchs O, Genuneit J, Karvonen AM, Hyvarinen A, Kaulek V, Roduit C, Weber J, Schaub B, Lauener R, Kabesch M, Pfefferle PI, Frey U, Pekkanen J, Dalphin JC, Riedler J, Braun-Fahrlander C, von Mutius E, Ege MJ, The PSG. Clinical and epidemiologic phenotypes of childhood asthma. American Journal of Respiratory and Critical Care Medicine 2013.
2. Fuchs O, von Mutius E. Prenatal and childhood infections: Implications for the development and treatment of childhood asthma. The Lancet Respiratory Medicine 2013;1:743-754.
3. Goldmann T, Pedersen F, Seehase S, Marwitz S, Lang DS, Kirsten AM, Zabel P, Vollmer E, Magnussen H, Rabe KF, Watz H. The effect of dithiothreitol on the transcriptome of induced sputum cells. Respiration; international review of thoracic diseases 2013;86:262-263.
4. Hagner S, Harb H, Zhao M, Stein K, Holst O, Ege MJ, Mayer M, Matthes J, Bauer J, von Mutius E, Renz H, Heine H, Pfefferle PI, Garn H. Farm-derived gram-positive bacterium staphylococcus sciuri w620 prevents asthma phenotype in hdm- and ova-exposed mice. Allergy 2013;68:322-329.
5. Hennig C, Ilginus C, Boztug K, Skokowa J, Marodi L, Szaflarska A, Sass M, Pignata C, Kilic SS, Caragol I, Baumann U, Klein C, Welte K, Hansen G. High-content cytometry and transcriptomic biomarker profiling of human b-cell activation. The Journal of Allergy and Clinical Immunology 2014;133:172-180 e171-110.
6. Kretschmer S, Dethlefsen I, Hagner-Benes S, Marsh LM, Garn H, Konig P. Visualization of intrapulmonary lymph vessels in healthy and inflamed murine lung using cd90/thy-1 as a marker. PloS One 2013;8:e55201.
7. Papi A, Corradi M, Pigeon-Francisco C, Baronio R, Siergiejko Z, Petruzzelli S, Fabbri LM, Rabe KF. Beclometasone-formoterol as maintenance and reliever treatment in patients with asthma: A double-blind, randomised controlled trial. The Lancet Respiratory Medicine 2013;1:23-31.
8. Pfefferle PI, Prescott SL, Kopp M. Microbial influence on tolerance and opportunities for intervention with prebiotics/probiotics and bacterial lysates. The Journal of Allergy and Clinical Immunology 2013;131:1453-1463; quiz 1464.

Chronic Obstructive Pulmonary Disease (COPD)

Disease Area Leaders	Prof. Dr. Klaus F. Rabe (ARCN) Prof. Dr. Claus F. Vogelmeier (UGMLC)
Participating DZL Partner Sites	ARCN, BREATH, CPC-M, TLRC, UGMLC
Number of Participating DZL Faculty	56

Chronic Obstructive Pulmonary Disease (COPD) is characterized by progressive and largely irreversible airflow limitation. Shortness of breath is the most common symptom of COPD and contributes significantly to the decreased quality of life experienced by many COPD patients. Although in part preventable, COPD is the 4th leading cause of death in the world. The most common causes of COPD are cigarette smoking and air pollution, and the most frequently encountered destructive lung disease is COPD linked to emphysema. Loss of structural integrity and regenerative capacity are critical for disease progression as well as for response or lack of response to therapy in COPD; however the underlying mechanisms remain poorly understood. The long term goal of the DZL COPD research effort is the translation of novel mechanism-based therapeutic concepts into effective therapies for COPD patients.



Goals followed in 2013

Goal 1 – Remodeling, regeneration and repair: from animal models to human tissues

- Development of conditional mouse models for chronic bronchitis and emphysema by regulated overexpression of ENaC in Clara cells and alveolar type II cells
- Identification of candidate genes through longitudinal phenotypic and molecular characterization of COPD mouse models
- Validation of candidate genes in native tissues and primary cultures of COPD
- Transcriptome analysis and target validation in human samples (sputum, lung tissue)

Goal 2 – Biomarkers and Phenotypes

- Biomarkers in exhaled breath and airway surface liquid
  - Development, improvement and standardization of sampling techniques for volatile molecules (VOC)
  - Analysis of VOCs after endotoxin provocation in healthy persons
  - Standardized collection of VOCs in COPD patients
  - VOC analysis of COPD cohorts
  - Identification and development of biomarkers in epithelial fluid by means of bronchoscopic microcollection and exhaled particle analysis
- Imaging Biomarkers
  - Development and adaptation of MRI sequences for the detection, quantification and monitoring of inflammatory airway changes
  - Determination of airway inflammation and local ventilation by MRI in healthy subjects after segmental endotoxin challenge
  - Determination of airway inflammation in COPD patients by MRI
  - MRI imaging in patients with COPD severity GOLD I - IV
- FRET-based sensors for quantitative monitoring of pulmonary inflammation and proteolysis
  - Development of sensitive and specific FRET sensors to determine the activity of pulmonary proteases (MMP12, neutrophil elastase, cathepsins)
  - Establishment of assays (FACS, microscopy) for FRET measurement in biosamples (sputum, BAL)
- Mucins
  - Development of mucin-reactive probes

Goal 3 – Measurement of physical activity

- Longitudinal measurement of activity
- Cross-sectional analyses

Goal 4 – Cohorts and clinical Studies

- Implementation of cohort studies
- Clinical trials in cooperation with industry partners
- Implementation of Investigator Initiated Trials after approval by the DZL “Clinical Trials Board”

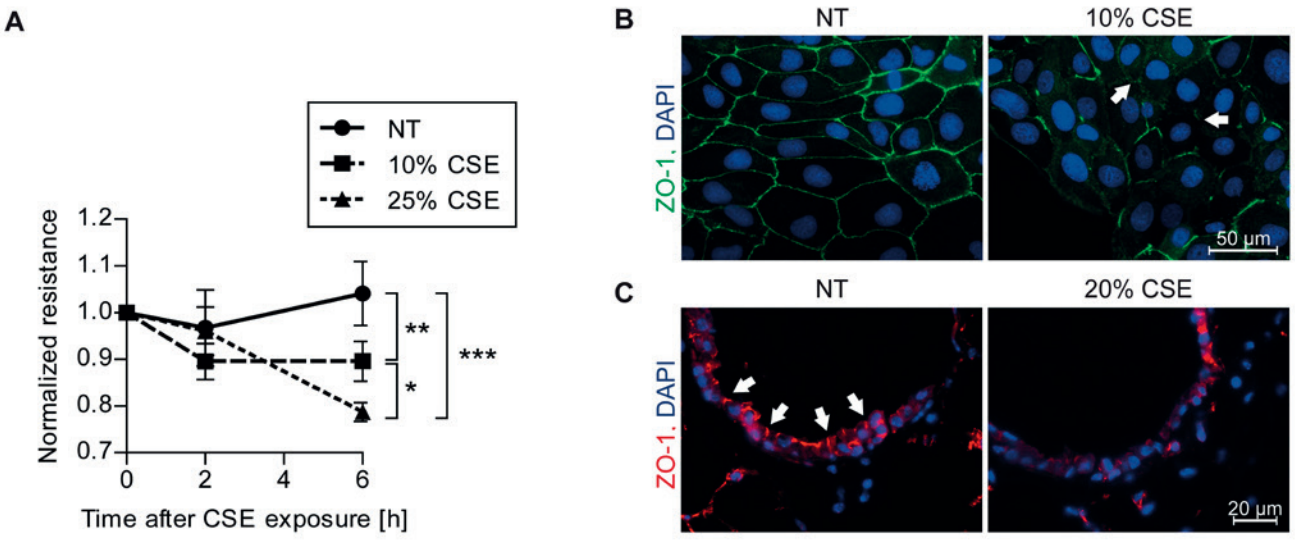
Goal 5 – Healthcare Management and Healthcare Economics

- Setup and piloting of the test network; evaluation of testing and of data quality control
- Recruitment of additional medical practices into the study, completion of the basic data set and conclusion of error analysis
- Further development of a cost-effective treatment model for COPD and introduction into the international COPD modelling group for model validation
- Healthcare economic analysis of cost and quality of life with respect to COPD risk factors (e.g., smoking)
- Piloting inquiry instruments for quality of life measurements

2013 Research Highlights – COPD

Research Highlight #1

Cigarette smoke extract (CSE) impairs the natural barrier function of bronchial epithelial cells:



The growth factor TGF-β1 counteracts the brochial barrier dysfunction after smoke injury:

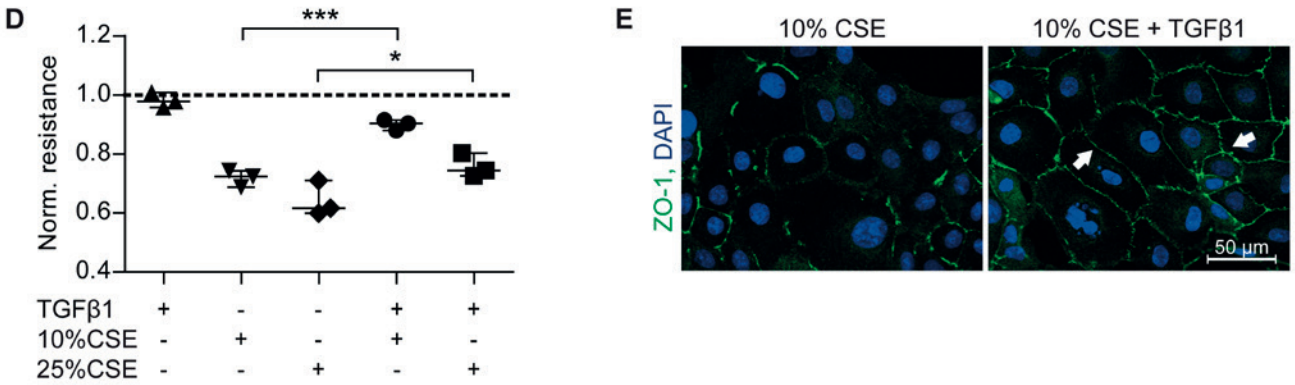


Figure 1. Cigarette smoke extract and its impact on natural barrier function of bronchial epithelial cells. *The airway epithelium constitutes an essential barrier to protect the body from inhaled insults, such as cigarette smoke. Since defective epithelial barrier function contributes to COPD, we investigated how cigarette smoke extract (CSE) affects bronchial epithelial barrier function and the cell-cell junctions – essential for the epithelial barrier. We showed that exposure of bronchial cells to CSE significantly decreased its barrier function (Fig. A) and diminished cell-cell junction components (here: tight junction protein ZO-1) in human bronchial epithelial cells (Fig. B) and isolated murine bronchi (Fig. C). Next, we examined how the growth factor TGF-β1 affects the bronchial epithelial barrier after smoke injury. We demonstrated for the first time that TGF-β1 co-treatment was able to prevent the smoke-induced loss in barrier function (Fig. D), accompanied by maintained cell-cell junctions (Fig. E). As such, TGF-β1 – known to be upregulated in different lung diseases – may serve as a protective factor for bronchial epithelial cell homeostasis early after injury. The reactivation of this protective pathway might represent a novel therapeutic approach in diseases such as COPD. (Figure adapted with permission of the American Thoracic Society. Copyright © 2014 American Thoracic Society. Schamberger et al., Am J Respir Cell Mol Biol 2014, 50:1040-1052. The AJRCMB is the official Journal of the American Thoracic Society.)*

Research Highlight #2: Assessment of airway inflammation by magnetic resonance imaging

We hypothesized that airway inflammation can be quantified by magnetic resonance imaging, correlates with cellular inflammation markers in bronchoalveolar lavage, and can be utilized as a novel biomarker for drug testing. DZL partners of the Platform Imaging and Disease Area COPD conducted a clinical trial in patients with asthma using segmental allergen challenge. Cellular inflammation markers were found to be closely correlated to semiquantitative changes (Turbo-Inversion Recovery-Magnitude magnetic resonance imaging for detection of local edema) and to quantitative changes (oxygen transfer function in T1-weighted images at 21% and 100% oxygen breathing) in magnetic resonance imaging. In addition, MRI allows multiple non-invasive assessments over time. Future work will focus on improvement of sensitivity to allow monitoring of airway inflammation by MRI in milder disease entities (Vogel-Claussen et al., Am J Respir Crit Care Med 2014; 189:650-7).

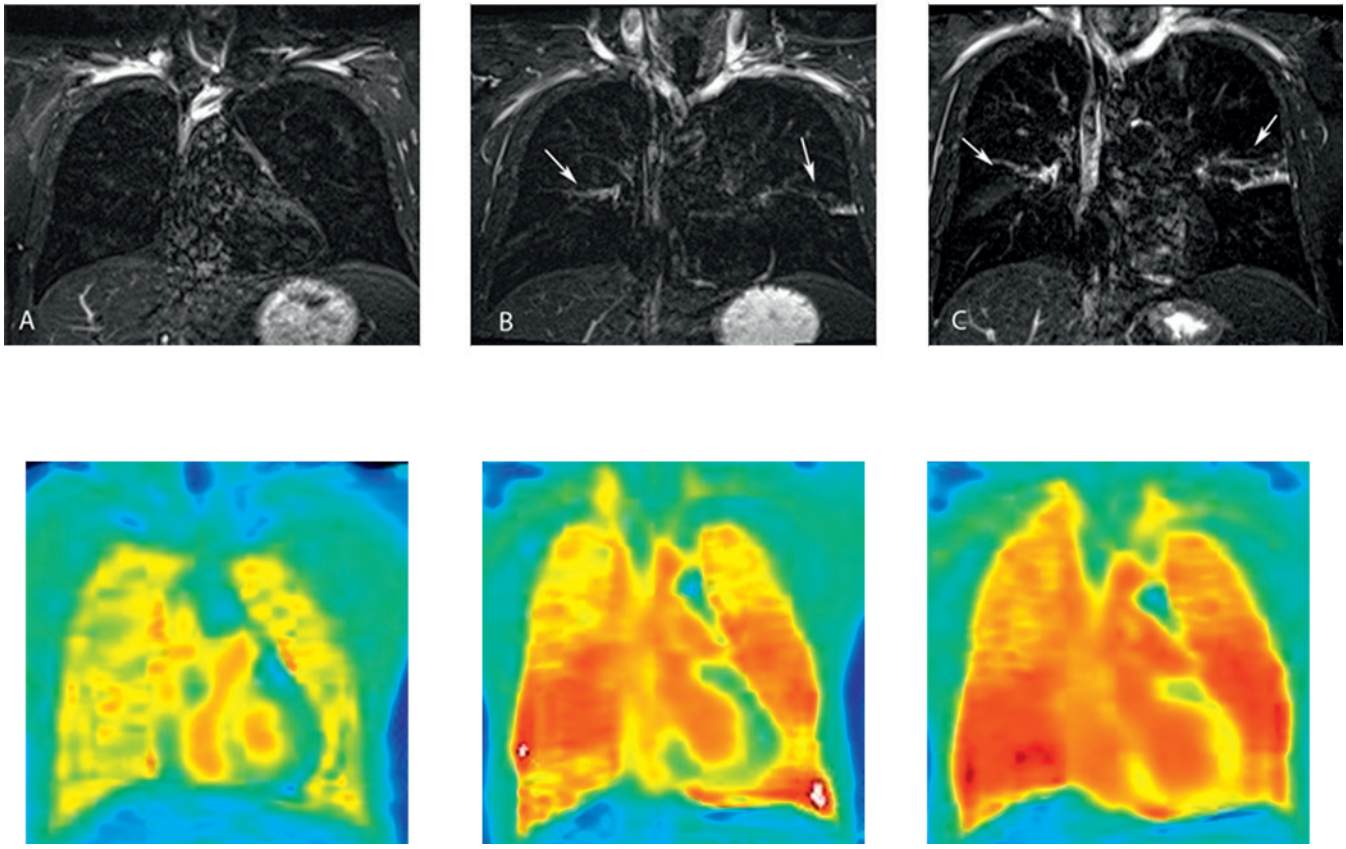


Figure 2. Magnetic resonance imaging of local inflammatory changes in a patient with asthma before (left images) as well as 6 hours (middle images) and 24 hours (right images) after segmental allergen challenge. *Upper panels show a TIRM sequence (Turbo-Inversion Recovery-Magnitude magnetic resonance imaging). The lower panels display T1-weighted intensity maps of oxygen transfer function. MRI changes correlate closely with cellular markers of inflammation in bronchoalveolar lavage.*

Research Highlight #3

This prospective trial aims to phenotype COPD by using imaging methods. The identification of different COPD phenotypes, such as the “emphysema-type” and the “airway-type” is important because therapy and prognosis will be different. Imaging might play a central role in diagnosing these phenotypes. So far computed tomography (CT) is regarded as the gold standard, but involves ionizing radiation and lacks functional information. The medical problem addressed in this trial (embedded in “ Competence Network Asthma and COPD, Cosyconet”) is the image-based phenotyping of COPD. The principal research question is whether magnetic resonance imaging (MRI) can replace CT for the characterization of COPD by “structural and functional phenotyping” on a regional basis. The sensitivity and specificity of MRI will be compared to Lowdose CT serving as the gold standard. To achieve this goal, MRI and CT of the lung will be performed in a multi-center cohort of 625 COPD patients from the main Cosyconet cohort. Organized and coordinated by the department of diagnostic and interventional Radiology of the Heidelberg University Hospital, member of the Platform Imaging and the TLRC, this Cosyconet subtrial (SP7) involves radiologic partners at all DZL sites. The infrastructure of the Cosyconet Image Bank (SP 5) which has been established to collect and analyze retrospective CT data from patients of the main cohort, will be used to perform the independent analysis and the software-based evaluations of the prospective MRI and CT image material of the subcohort.

The trial started in December 2013 with successful enrollment of the first patient at the University Hospital Heidelberg.

Cosyconet – Functional Imaging & Image Bank

Image-Based Structural and Functional Phenotyping using MRI and CT

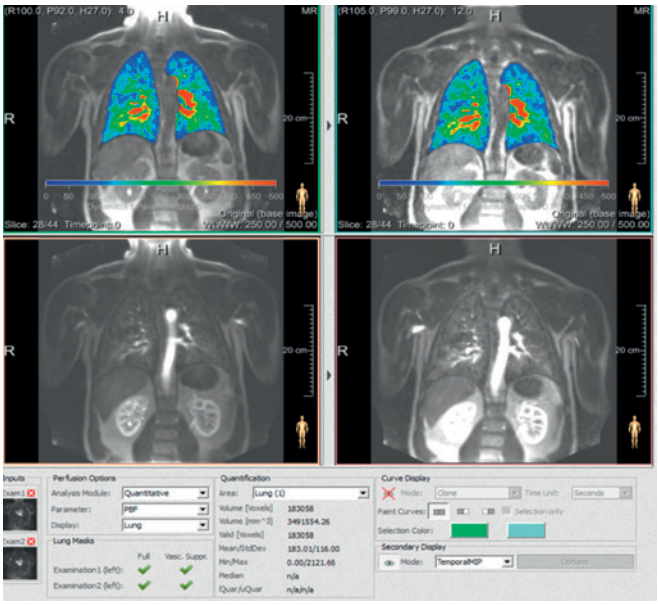


Fig. 3A: Quantitative Analysis of Perfusion-MRI “Pulmo-MR” (MeVis/Bremen)

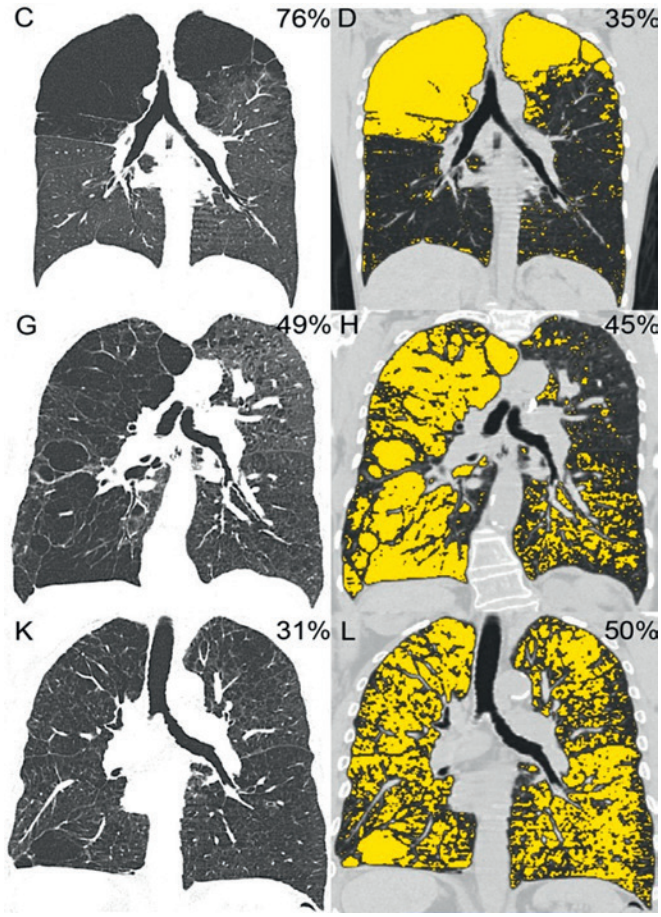
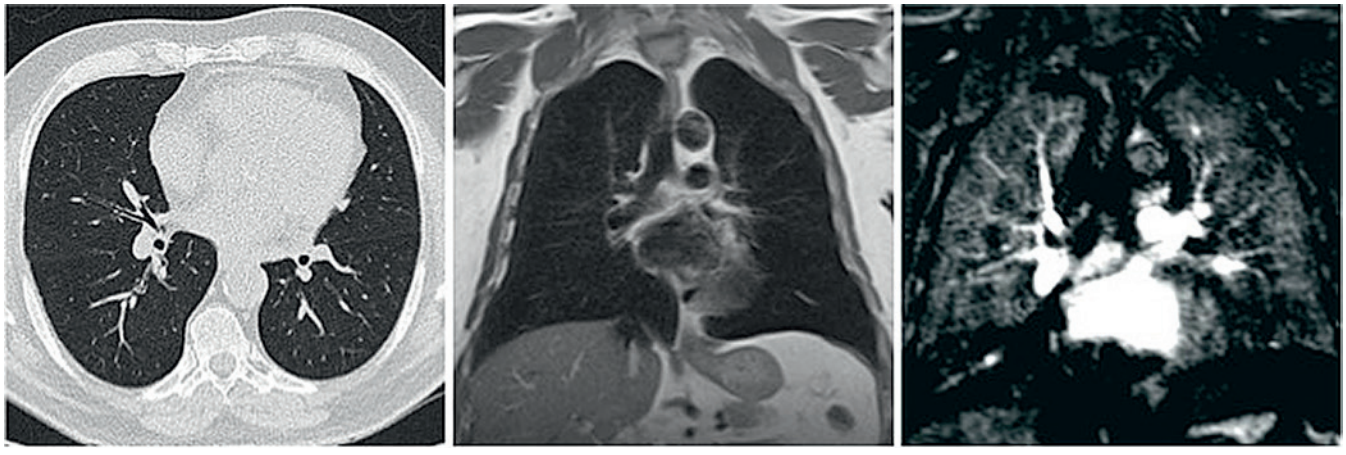


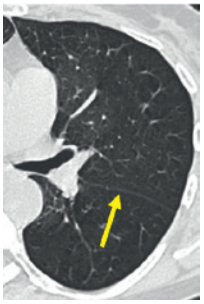
Fig. 3B: Quantitative CT-Evaluation "Yacta" (TLRC)

Fig. 3C: 68 year old patient (GOLD stage II) with airway wall thickening and mild centrilobular emphysema (visible on CT), normal appearance of the pulmonary parenchyma on T2w-HASTE, and perfusion deficits on DCE perfusion MRI



Research Highlight #4: Pulmonary imaging for endoscopic treatment

Endoscopic lung volume reduction (ELVR) therapy of selected hyperinflated lobes improves lung function, exercise tolerance and prolongs life in patients with severe emphysema. Endobronchial valves (EBV), that allow air to escape from but not to enter in selected lobes, have been used for ELVR, which ensures a better supply to healthy lung areas with breathing air. It has been observed that the efficacy of a lung volume reduction with endobronchial valves is weakened in some patients with severe emphysema because of collateral ventilation pathways between the pulmonary lobes (parenchymal bridges). The presence of collateral ventilation might correlate with the extent of pulmonary fissures. In two retrospective studies conducted in cooperation of the Disease Area COPD and the Platform Imaging at the TLRC, it was recently demonstrated that patients with heterogeneous, upper predominant emphysema and complete fissures submitted to EBV-therapy have a greater probability of clinical improvement. Computed tomography is the best method for assessing emphysema distribution, pulmonary anatomy, and fissure integrity when evaluated by dedicated chest radiologists. (References: Koenigkam-Santos M et al., Eur J Radiol. 2013;82:2365-70 und Koenigkam-Santos et al., Radiol Bras. 2013;46:15–22.)



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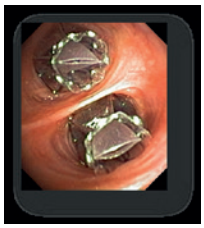

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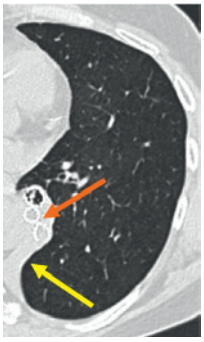

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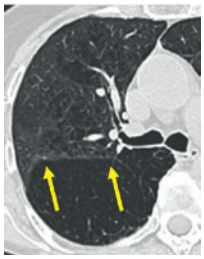

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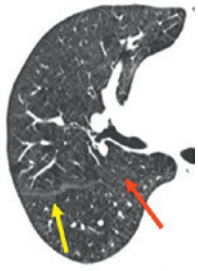


E

Figures 4A, B, C: A patient with marked emphysema of the left lower lobe, with a complete interlobar fissure *(A). The left lower lobe was treated with endoscopic lung volume reduction (ELVR), which means that valves were placed into the segmental bronchi (B), with a consecutive complete atelectasis of the lobe (C).*

Figures 4 D, E: A patient with emphysema and hyperinflation of the right lower lobe, which may serve as a potential target for ELVR *(D). A detailed analysis of the interlobar fissures revealed, however, an incomplete fissure as a risk for collateral ventilation, thus excluding the lung from occluding ELVR (E). Non-occluding options (polymeric, caloric, Coils, OP) were evaluated.*

Research Highlight #5: Aging, lung function and disease

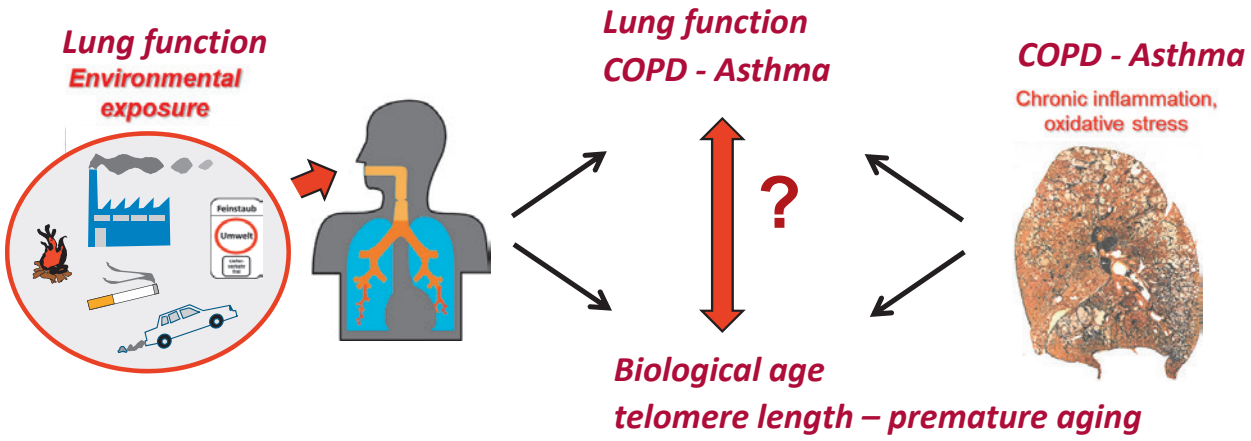


Figure 5. The relationship of telomere length with lung function. *Increased life expectancy is associated with increased susceptibility for prevailing chronic diseases, such as cardiovascular diseases, diabetes or cancer, suggesting that common, age-related processes are involved in these diseases. To test whether this finding is also true for two major chronic lung diseases, asthma and chronic obstructive disease (COPD), we studied the relationship of telomere length, as a marker of biological age, with lung function and respiratory disease in a large population based sample of up to 30,000 subjects. We observed shorter telomeres in patients with COPD and asthma compared to healthy controls, corresponding to an increased biological age of these patients; thus, our results provide evidence that premature aging and cell senescence contribute to the pathobiology of these chronic lung diseases. Further, we observed a positive association between telomere length and lung function indicating that younger biological age is associated with better lung function. This finding suggests that lung function partially reflects biological aging and contributes to the large variability of lung function observed between individuals (Albrecht et al., Eur Respir J 2014; 43: 983–992).*

Number of papers published by DZL Faculty in 2013 – Disease Area COPD: 57

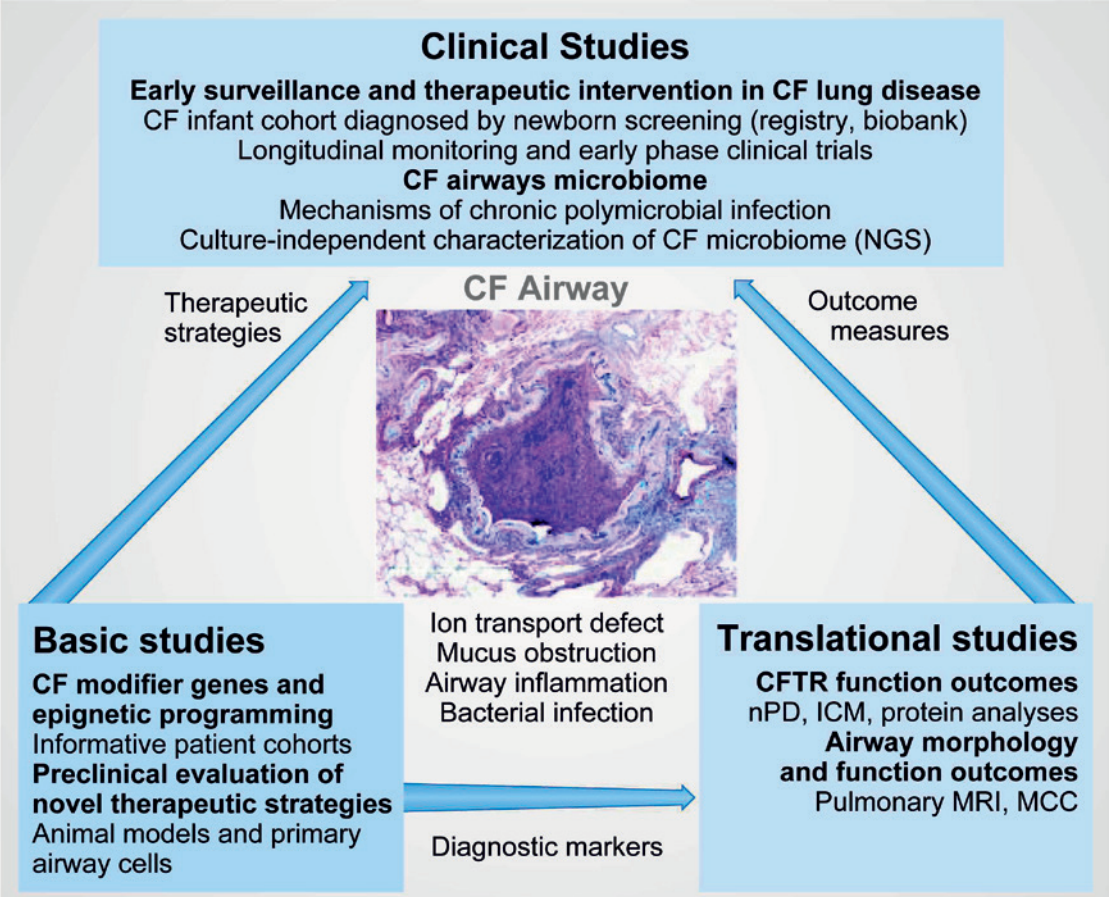
Highlighted Publications

1. Albrecht E, Sillanpaa E, Karrasch S, Alves AC, Codd V, Hovatta I, Buxton JL, Nelson CP, Broer L, Hagg S, Mangino M, Willemsen G, Surakka I, Ferreira MA, Amin N, Oostra BA, Backmand HM, Peltonen M, Sarna S, Rantanen T, Sipila S, Korhonen T, Madden PA, Gieger C, Jorres RA, Heinrich J, Behr J, Huber RM, Peters A, Strauch K, Wichmann HE, Waldenberger M, Blakemore AI, de Geus EJ, Nyholt DR, Henders AK, Piirila PL, Rissanen A, Magnusson PK, Vinuela A, Pietilainen KH, Martin NG, Pedersen NL, Boomsma DI, Spector TD, van Duijn CM, Kaprio J, Samani NJ, Jarvelin MR, Schulz H. Telomere length in circulating leukocytes is associated with lung function and disease. The European Respiratory Journal 2013.
2. Kirsten A, Watz H, Pedersen F, Holz O, Smith R, Bruin G, Koehne-Voss S, Magnussen H, Waltz DA. The anti-il-17a antibody secukinumab does not attenuate ozone-induced airway neutrophilia in healthy volunteers. The European Respiratory Journal 2013;41:239-241.
3. Koenigkam-Santos M, de Paula WD, Owsijewitsch M, Wielputz MO, Gompelmann D, Schlemmer HP, Kauczor HU, Heussel CP, Puderbach M. Incomplete pulmonary fissures evaluated by volumetric thin-section ct: Semi-quantitative evaluation for small fissure gaps identification, description of prevalence and severity of fissural defects. European Journal of Radiology 2013;82:2365-2370.
4. Koenigkam-Santos M, de Paula WD, Gompelmann D, Kauczor HU,Heussel CP, Puderbach M. Endobronchial valves in severe emphysematous patients: CT evaluation of lung fissures completeness, treatment radiological response and quantitative emphysema analysis. Radiol Bras. 2013; 46:15–22
5. Rabe KF, Fabbri LM, Vogelmeier C, Kogler H, Schmidt H, Beeh KM, Glaab T. Seasonal distribution of copd exacerbations in the prevention of exacerbations with tiotropium in copd trial. Chest 2013;143:711-719.
6. Schamberger AC, Mise N, Jia J, Genoyer E, Yildirim AO, Meiners S, Eickelberg O. Cigarette smoke-induced disruption of bronchial epithelial tight junctions is prevented by transforming growth factor-beta. American Journal of Respiratory Cell and Molecular Biology 2014; 50:1040-1052.
7. Vogel-Claussen J, Renne J, Hinrichs J, Schonfeld C, Gutberlet M, Schaumann F, Winkler C, Faulenbach C, Krug N, Wacker FK, Hohlfeld JM. Quantification of pulmonary inflammation after segmental allergen challenge using turbo-inversion recovery-magnitude magnetic resonance imaging. American Journal of Respiratory and Critical Care Medicine 2014;189:650-657.
8. Watz H, Barnacle H, Hartley BF, Chan R. Efficacy and safety of the p38 mapk inhibitor losmapimod for patients with chronic obstructive pulmonary disease: A randomised, double-blind, placebo-controlled trial. The Lancet Respiratory Medicine 2014;2:63-72.

Cystic Fibrosis

Disease Area Leaders	Prof. Dr. Marcus Mall (TLRC) Prof. Dr. Dr. Burkhard Tümmler (BREATH)
Participating DZL Partner Sites	ARCN, BREATH, TLRC, UGMLC
Number of Participating DZL Faculty	21

Cystic fibrosis (CF) is the most common genetically determined, early onset and still lethal form of chronic obstructive lung disease. CF affects approximately one in 2500 newborns in Caucasian populations. With improvements in symptomatic therapies and standardized CF medical care, the median survival of CF patients in Germany has increased to approximately 40 years of age. However, despite recent breakthroughs in disease-modifying therapies for a small subgroup of patients with specific CF genotypes, there are currently no therapies available that target CF lung disease at its root cause in the majority of patients. The overall aim of the DZL CF research program is to advance the current understanding of the pathogenesis of CF lung disease and to use this knowledge to improve CF diagnostics, develop more sensitive tools for monitoring of disease activity, and develop novel strategies for effective prevention and therapy of CF lung disease.



Disease Area Cystic Fibrosis Research Strategy

Goals Followed in 2013 – Cystic Fibrosis

Goal 1 – Basic CF Research: From Modifiers to Novel Therapeutic Targets

- Genetic modifiers of CF Lung Disease
  - Identification of disease modifying genes in a mouse model of CF lung disease
  - Identification of disease modifying genes in CF sibling pairs
- Epigenetic Programming of CF Lung Disease
  - Sequencing of immunoglobulin and T-cell receptor genes in monozygotic (identical) twins with CF
- Preclinical evaluation of mucolytic and anti-inflammatory treatment strategies
  - Preclinical evaluation of rehydrating and mucolytic strategies (hypertonic saline, long-acting sodium channel blocker) in ß ENaC overexpressing mice
  - Preclinical evaluation of DNAzymes to correct the ion transport defect in ß ENaC overexpressing mice

Goal 2 – Translational CF Research: Biomarkers and Outcome Measures

- Monitoring CFTR function ex vivo and in vivo
  - Standardization and evaluation of functional CFTR and biochemical analysis (nPD, ICM and CFTR immunoblots)
  - Evaluation and use of the CFTR analysis (nPD, ICM and CFTR immunoblots) to improve the CF diagnosis
- Morphology and function of the respiratory system: pulmonary MRI and mucociliary clearance
  - Development and evaluation of morphological and functional MRI scores for non-invasive diagnostic monitoring of CF lung disease
  - Evaluation of lung MRI as a new endpoint in clinical trials (interventions: antibiotics, physiotherapy, inhaled mucolytics)
  - Application of lung MRI for longitudinal study of lung disease in CF newborn screening cohort

Goal 3 – Clinical CF Research Programs

- Disease surveillance and therapeutic intervention in early CF lung disease
  - Establishment and validation of biochemical neonatal screening for CF
  - Building a cohort of newborn screening in early diagnosed CF patients
  - Longitudinal studies of early changes and spontaneous course of lung disease in the CF newborn screening cohort
- The Microbiome of CF Airways
  - Investigation of the microbiome of the upper and lower airways of CF patients using culture-independent methods before, during and after pulmonary exacerbation

2013 Research Highlights – Cystic Fibrosis

Research Highlight #1

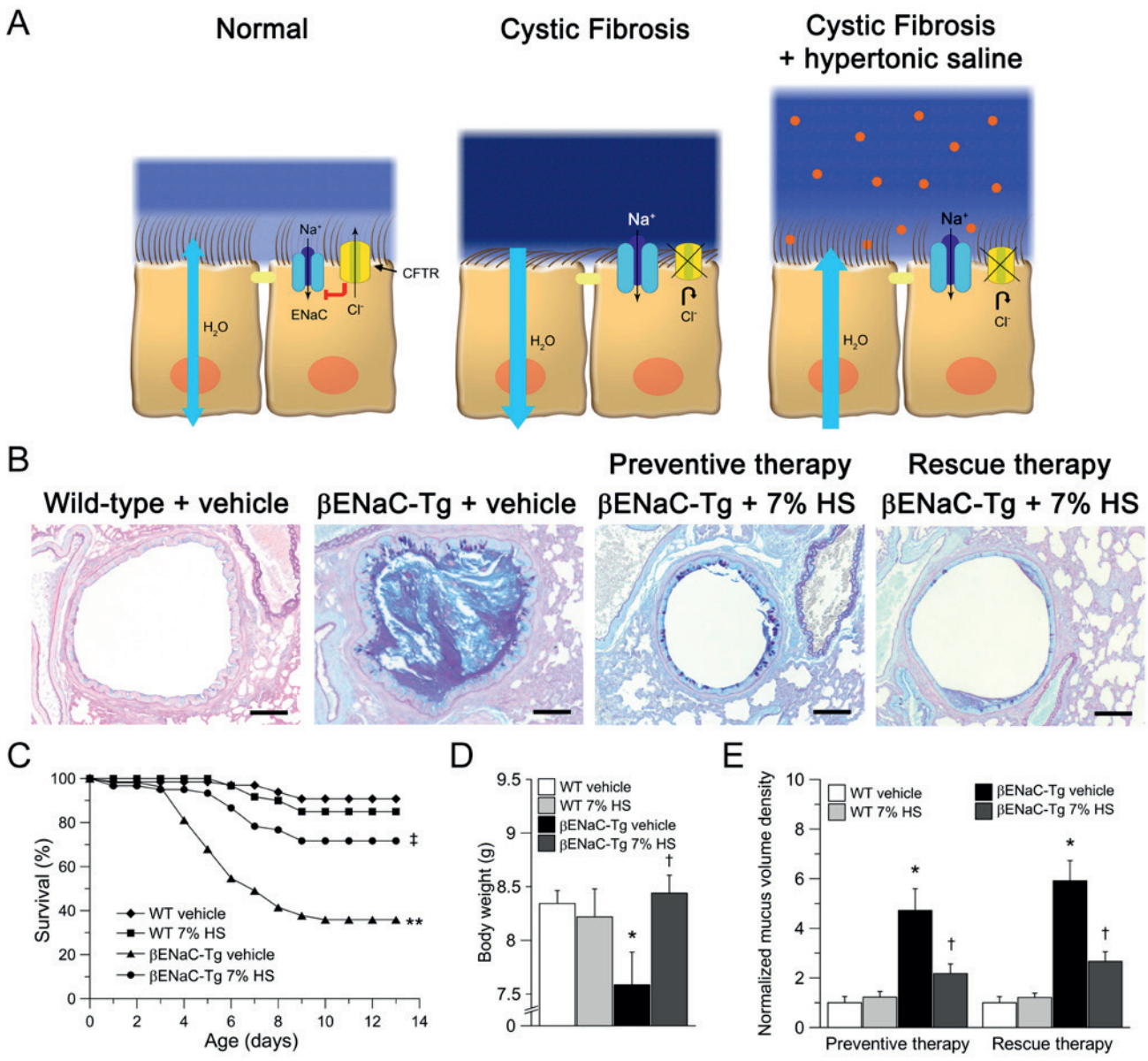


Figure 1. Airway surface dehydration causes mucus plugging in patients with cystic fibrosis (CF). *In a mouse model of CF lung disease we could show that preventive rehydration therapy with hypertonic saline is an effective mucolytic therapy that reduces mucus obstruction. Reduction of airway mucus by hypertonic saline improves survival and reduces failure to thrive in mice with CF-like lung disease. Further, we demonstrate that hypertonic saline is an effective rescue therapy to reduce airway mucus in mice with chronic airway disease. These results suggest that therapy with inhaled hypertonic saline has a high potential for the prevention of mucus obstruction in infants and young children with CF. Further, patients with other chronic obstructive lung diseases, such as COPD, may benefit from mucus-reducing effects of hypertonic saline (Graeber SY, Zhou-Suckow Z, Schatterny J, Hirtz S, Boucher RC, Mall MA. Hypertonic saline is effective in the prevention and treatment of mucus obstruction, but not airway inflammation, in mice with chronic obstructive lung disease. Am J Respir Cell Mol Biol 2013; 49:410-417.).*

Research Highlight #2

The major cystic-fibrosis causing mutation F508del is a 3-bp in-frame deletion of the phenylalanine codon F508 of the CFTR gene. The consequence of the deletion is impaired processing and transport of the protein from the endoplasmic reticulum (ER) to the apical membrane of the epithelial cell. However, in a minority of subjects with cystic fibrosis who are homozygous for the F508del mutation, mature, and functional F508del CFTR is produced in clinically meaningful amounts. Starting from mapping information data gained by the North American Cystic Fibrosis Modifier Consortium that the region 11p13 on chromosome 11 encodes an important modifier of cystic fibrosis disease, researchers at DZL have now identified genetic variants of the transcription factor EHF locus at 11p13 as a key modifier of the fate of F508del CFTR in the patient's epithelium (Stanke et al., 2013).

The figure depicts the EHF-dependent gene regulation in relation to F508del-CFTR biosynthesis, trafficking and posttranslational modification. In subpanel (a) mature, fully glycosylated and functional F508del-CFTR – emphasized as a green line at the apical membrane (AM) – reaches the apical membrane through trafficking pathways illustrated as green arrows. In contrast, pathways that lead to degradation of F508del-CFTR are depicted in orange. F508del-CFTR homozygous carriers of at least one frequent EHF allele exhibited no CFTR activity and upregulated the expression of genes in their epithelial tissue (shown in orange) that facilitate the degradation of F508del CFTR. Conversely, F508del-CFTR homozygous carriers of two rare EHF alleles exhibited residual CFTR activity of chloride secretion and upregulated the expression of genes in their epithelial tissue (shown in green) that facilitate the trafficking and maturation of F508del CFTR. EHF-dependent differentially regulated genes that are crucial for F508del-CFTR biosynthesis, maturation and trafficking are shown in subpanels (b) to (j). Our study provides another important cornerstone for understanding the origins of CF phenotypes.

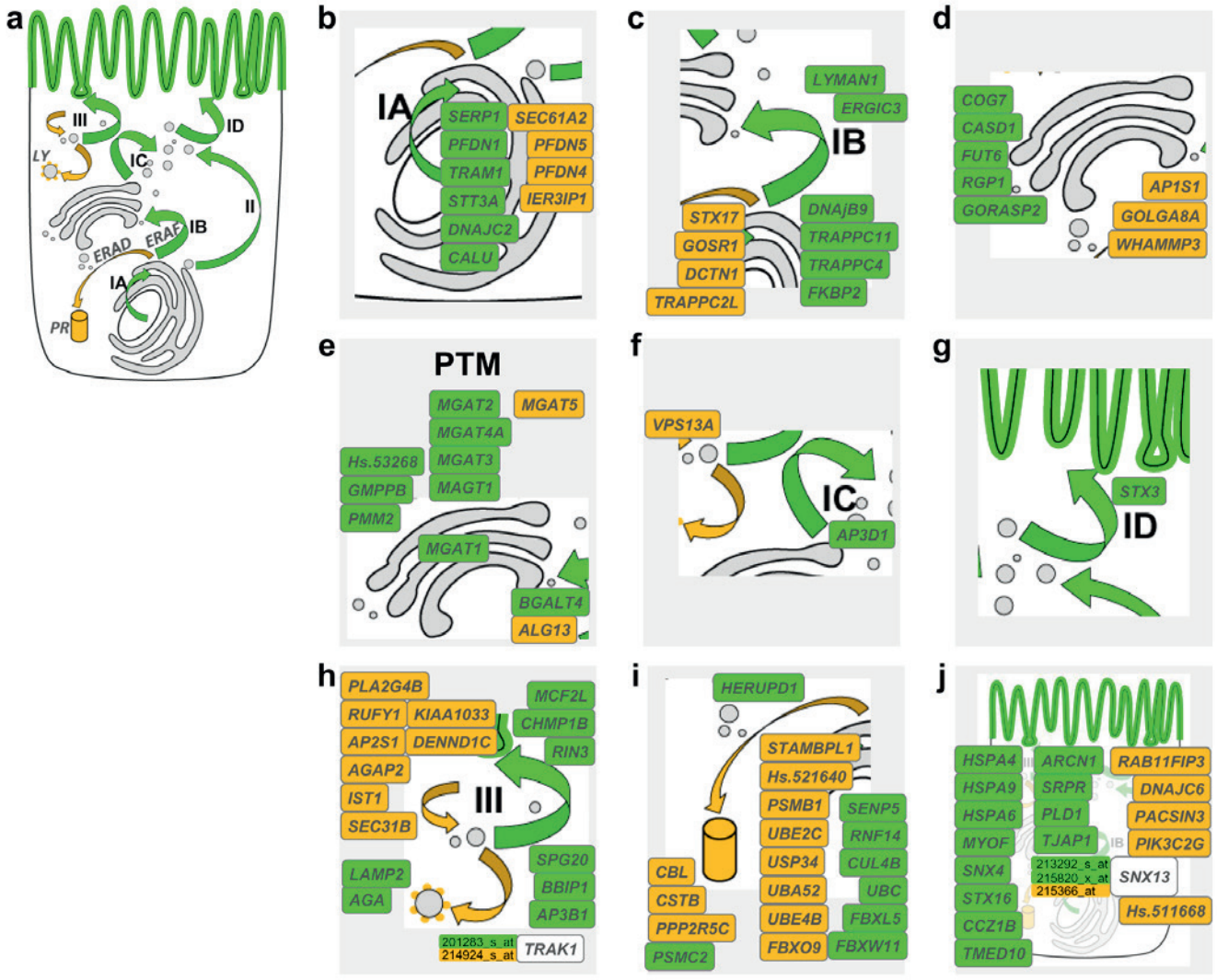


Figure 2 EHF-dependent gene regulation in relation to F508del-CFTR biosynthesis, trafficking and posttranslational modification.

a Mature, fully glycosylated and functional p.Phe508del-CFTR – emphasized as a green line at the apical membrane (AM) – reaches the apical membrane through trafficking pathways illustrated as green arrows. These encompass the ER-associated degradation pathway (ERAD) that leads to degradation in the proteasome (PR) and the retrograde traffic of endosomes from the subapical compartment (III) towards the lysosome (LY).

b–j EHF-dependent differentially regulated genes whose products that have been annotated to partake in any of these pathways crucial to p.Phe508del-CFTR biosynthesis, maturation and trafficking. Gene products for which the subcellular localization cannot be specified are listed in (j). Forty trafficking and maturation genes whose expression are upregulated among the nine p.Phe508del-CFTR homozygous carriers of at least one frequent EHF allele are shown in orange. Fifty-eight trafficking and maturation genes whose expression are upregulated among the seven p.Phe508del-CFTR homozygous carriers of two rare EHF alleles are shown in green. (Reference: Stanke F, van Barneveld A, Hedtfeld S, Wölfl S, Becker T, Tümmler B. The CF-modifying gene EHF promotes p.Phe508del-CFTR residual function by altering protein glycosylation and trafficking in epithelial cells. Eur J Hum Genet. 2013 Oct 9. doi: 10.1038/ejhg.2013.209.)

Research Highlight #3

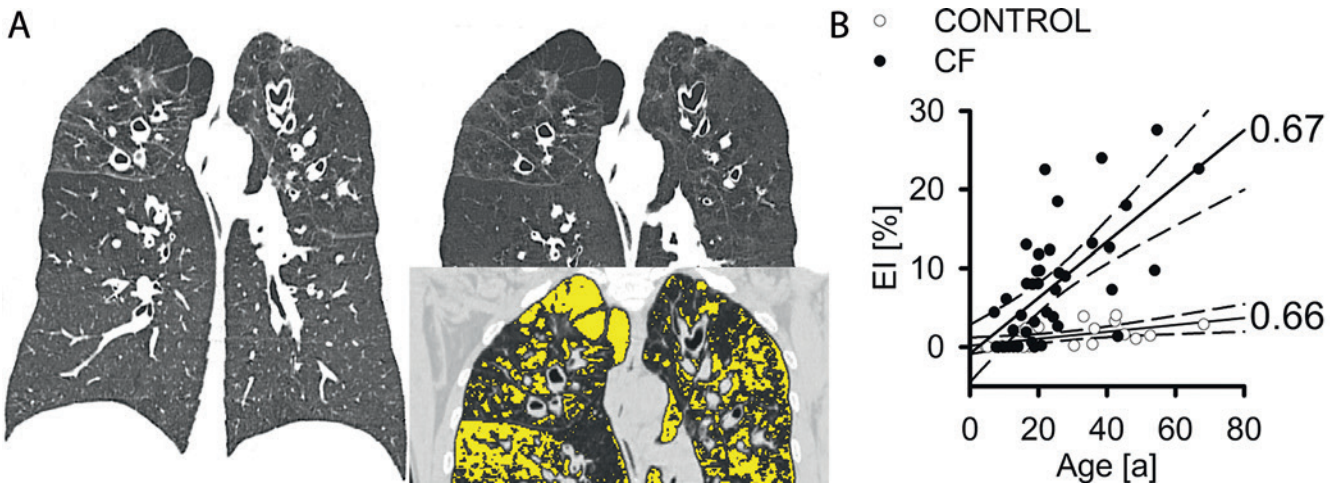


Figure 3. Development of emphysema is characteristic for lung disease in patients with CF. *In a previous study using volumetric computer tomography in an animal model of CF lung disease (the transgenic βENaC mouse), we were able to show that in addition to chronic airway disease there is also evidence of lung parenchymal tissue damage that resembles emphysema (Wielpütz MO et al. Eur Respir J 2011). So far, emphysema had been associated with smoking-induced chronic obstructive pulmonary lung disease (COPD) but not with CF. Based on these basic research results we were able to demonstrate that the development of emphysema is also characteristic for lung disease in patients with CF. We employed a newly developed software tool to determine lung density (in Hounsfield units) based on volumetric high resolution computed tomography data of the chest and compared the lung density between CF patients and healthy controls. Figure (A) provides an example of lung tissue affected by emphysema highlighted in yellow. In figure (B), the emphysema index (EI, proportion of emphysema in relation to total lung volume) is plotted against the age of CF patients and healthy controls. These results demonstrate that emphysema is frequently observed from adolescence onwards in patients with CF. Further, we found that the presence of emphysema was associated with more severe impairment of lung function in CF. The software for the quantification of emphysema severity is now available for routine clinical use. By using this technology we can improve monitoring of CF lung disease and the new insights gained may improve individualized therapeutic approaches for lung disease in patients with CF. (Figure modified from Wielpütz MO, Weinheimer O, Eichinger M, Wiebel M, Biederer J, Kauczor HU, Heussel CP, Mall MA, Puderbach M. Pulmonary emphysema in cystic fibrosis detected by densitometry on chest multidetector computed tomography. PLOS One 2013;8:e73142.)*

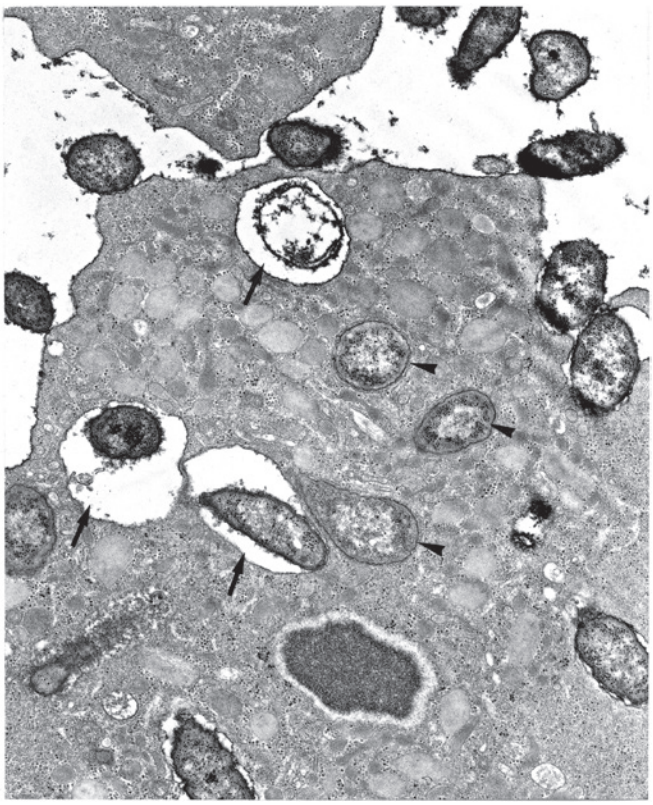
Figure 4 (left). Phagocytosis test with TBCF10839 (108 cfu ml-1, 107 PMN ml-1, t = 2 min; magnification 14 000×). *Extracellular bacteria are covered with a matrix stained with ruthenium red. Within PMNs bacteria reside in phagolysosomes (arrows) and free in the cytosol (arrowheads).*

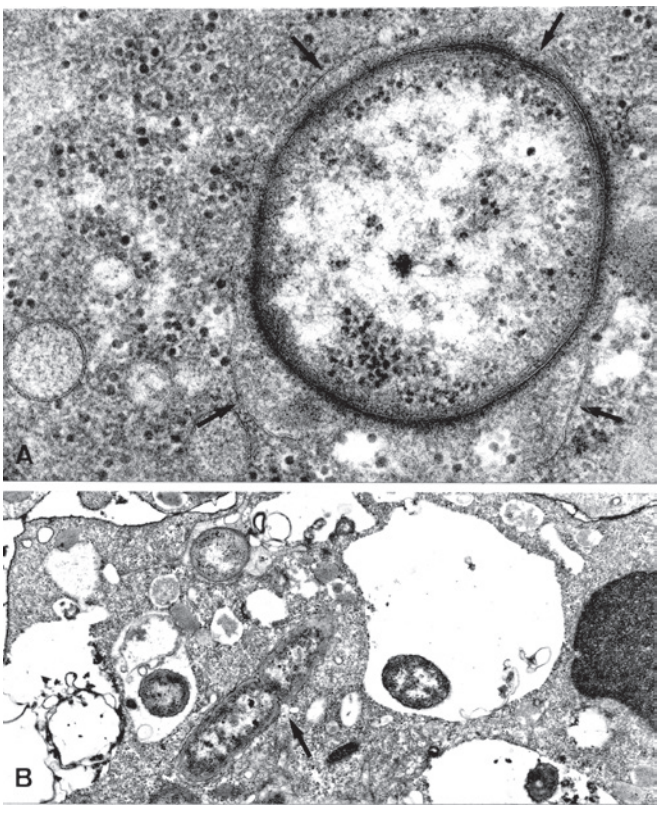
Figure 4 (right) Phagocytosis test with TBCF10839 *(108 cfu ml-1, 107 PMN ml-1). A. Disintegration of the phagolysosomal membrane (arrows). The double membrane of the bacterium is intact (t = 5 min; magnification 48 000×). B. Division of TBCF10839 (arrow) in a devitalized PMN (t = 120 min; magnification 10 000×).*

Klockgether J, Miethke N, Kubesch P, Bohn YS, Brockhausen I, Cramer N, Eberl L, Greipel J, Herrmann C, Herrmann S, Horatzek S, Lingner M, Luciano L, Salunkhe P, Schomburg D, Wehsling M, Wiehlmann L, Davenport CF, Tümmler B. Intraclonal diversity of the Pseudomonas aeruginosa cystic fibrosis airway isolates TBCF10839 and TBCF121838: distinct signatures of transcriptome, proteome, metabolome, adherence and pathogenicity despite an almost identical genome sequence. Environ Microbiol 2013; 15:191-210.

Research Highlight #4

Chronic lung infections with Pseudomonas aeruginosa bacteria are a major cause of disease complications in patients with cystic fibrosis (CF). DZL researchers performed the first comprehensive comparison of both genomes and phenotypes of P. aeruginosa CF airway isolates from unrelated patients (Klockgether et al., 2013). The two patients had acquired the so-called TB clone from the same source, and at the time of isolation the Pseudomonas bacteria had persisted in the patients' airways for about two years. Whereas genomes of unrelated P. aeruginosa strains differ by about 50,000 nucleotide substitutions from each other, less than ten differences were detected between the genomes of these two closely related strains. Despite their almost identical genome sequence, the two clone TB strains strongly differed in their global gene expression and metabolite profiles and their airway colonization capacity. Even more spectacular, one strain was susceptible to killing by leukocytes as it is typically observed for all P. aeruginosa, whereas the other strain could persist and replicate in neutrophilic leukocytes that are man's major antipseudomonal weapon. During colonization of the CF patient's airways a single intragenic deletion in a key gene had transformed a moderately pathogenic Pseudomonas bacterium into a highly virulent intracellular pathogen. Fortunately, this particular strain could be eliminated by targeted antibiotic therapy. Microevolution of P. aeruginosa in CF lungs can generate novel complex traits by few or even single mutations.





Number of papers published by DZL Faculty in 2013 - Disease Area Cystic Fibrosis: 22

Highlighted Publications

1. Davenport CF, Tummler B. Advances in computational analysis of metagenome sequences. Environmental Microbiology 2013;15:1-5.
2. Graeber SY, Zhou-Suckow Z, Schatterny J, Hirtz S, Boucher RC, Mall MA. Hypertonic saline is effective in the prevention and treatment of mucus obstruction, but not airway inflammation, in mice with chronic obstructive lung disease. American Journal of Respiratory Cell and Molecular Biology 2013;49:410-417.
3. Klockgether J, Miethke N, Kubesch P, Bohn YS, Brockhausen I, Cramer N, Eberl L, Greipel J, Herrmann C, Herrmann S, Horatzek S, Lingner M, Luciano L, Salunkhe P, Schomburg D, Wehsling M, Wiehlmann L, Davenport CF, Tummler B. Intraclonal diversity of the pseudomonas aeruginosa cystic fibrosis airway isolates tbcf10839 and tbcf121838: Distinct signatures of transcriptome, proteome, metabolome, adherence and pathogenicity despite an almost identical genome sequence. Environmental Microbiology 2013;15:191-210.
4. Stanke F, van Barneveld A, Hedtfeld S, Wolfl S, Becker T, Tummler B. The cf-modifying gene ehf promotes p.Phe508del-cftr residual function by altering protein glycosylation and trafficking in epithelial cells. European Journal of Human Genetics : EJHG 2014;22:660-666.
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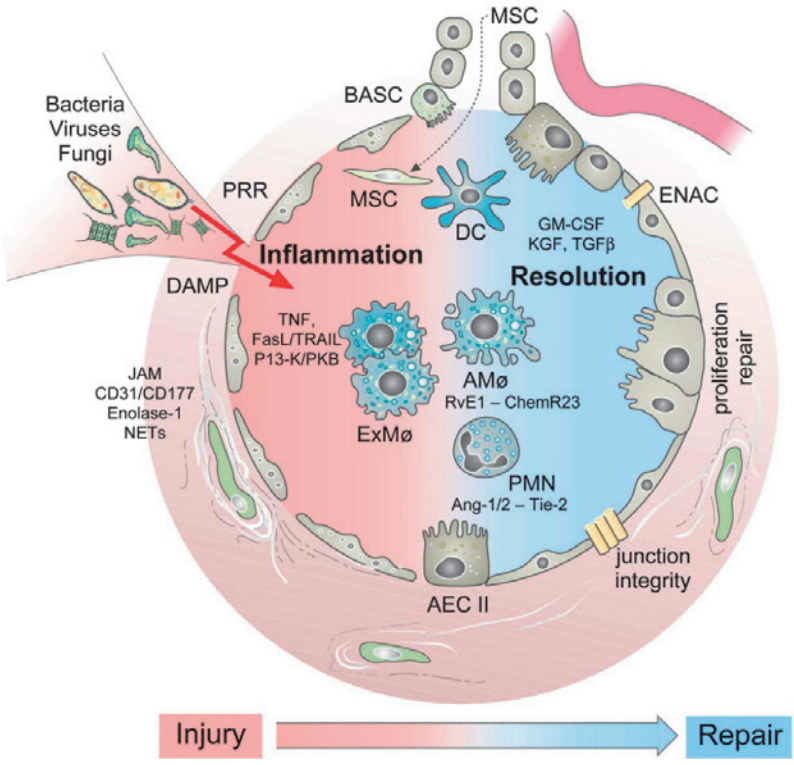
Pneumonia And Acute Lung Injury

Disease Area Leaders	Prof. Dr. Jürgen Lohmeyer (UGMLC) Prof. Dr. Tobias Welte (BREATH)
Participating DZL Partner Sites	ARCN, BREATH, CPC-M, TLRC, UGMLC
Number of Participating DZL Faculty	27

Acute lower respiratory tract infections represent an increasing public health problem worldwide, resulting in a disease burden greater than that of any other infection with mortality rates unchanged over the past 50 years. Likewise, the lack of any pharmacological treatment for the most devastating clinical course of pulmonary infection, the acute respiratory distress syndrome (ARDS), coupled with an unacceptably high mortality rate, underscores an urgent medical need for novel, effective therapeutic approaches. The DZL aims to dissect the molecular mechanisms underlying the sensing of, and signaling in response to microbial insult and inflammatory infiltration with the aim of developing targeted interventions to attenuate lung injury in pneumonia and ARDS.

Both microbial attack (bacteria, viruses, fungi) and non-microbial inflammatory injury (aspiration, toxic gases) may cause acute lung injury with severe respiratory failure. The goal of this Disease Area is to dissect the molecular mechanisms underlying the spreading of inflammatory events in the alveolar compartments, and to understand the cellular and molecular players driving resolution of inflammation and repair of alveolar integrity. Based on understanding these events, new therapeutic concepts are to be developed.



(AEC II – alveolar type II cells; AMø – alveolar macrophage; Ang – angeopoietin; BASC – bronchioalveolar stem cells; DAMP – damage-associated molecular patterns; DC – dendritic cell; ENAC – epithelial socium channel; ExMø – exudate macrophages; FasL – Fas-ligand; FGF – Fibroblast Growth Factor; GM-CSF - granulocyte macrophage colony-stimulating factor; JAM – junctional adhesion molecule; BETs – neutrophil extracellular traps; KGF – keratinocyte growth factor; MSC – mesenchymal stem cell; PI3K – phosphatidylinositol-3 kinase; PKB – protein kinase B; PNM – neutrophils; PRR – pattern recognition receptors; RvE1 – resolvin; TGF – transforming growth factor; TNF – tumor necrosis factor; TRAIL – TNF-related apoptosis inducing ligand)

Goals Followed in 2013 – Pneumonia and Acute Lung Injury

Goal 1 - Sensing Microbial and Inflammatory Lung Attack

- Basic Research
  - Characterization of pulmonary pattern recognition molecules for pathogen/host ligands
  - Identification of "Immune Escape" strategies of pulmonary pathogens
  - Evaluation of the role of 'Brush cells' as sensors of microbial pathogens in the bronchial tree
- Translational Research
  - Characterization of pulmonary host defense of wild type and C-type Lectin receptor (CLR) deficient mice in focal pneumonia
  - Preclinical evaluation of further pulmonary pattern recognition molecules as potential targets for therapeutic intervention
  - Employment of Pattern Recognition Receptors on dendritic cells for immunomodulation
- Clinical Research
  - Creation of BAL inflammatory profiles in pneumonia/ARDS patient cohorts

Goal 2 - Lung Innate Immune Responses

- Basic Research
  - Analysis of pathogen-specific pulmonary recruitment of inflammatory cells in pneumonia/ARDS
  - Analysis of conditional mutant mice with lung cell type specific gene targeting
- Translational Research
  - Establishment of the lung-specific transient over-expression of macrophage-related chemokines in the mouse
  - Analysis of effector cell function resident macrophages in the presence and absence of overexpressed pulmonary cytokines
- Clinical Research
  - Evaluation of molecular inflammatory signatures for individualized pneumonia/ARDS therapy

Goal 3 – Resolution of Lung Inflammation, Lung Barrier Protection and Regeneration

- Basic Research
  - Investigation of the influence of the pulmonary inflammation processes by local hypoxia, endocrine signals and the type of ion transport
  - Establishment of intervention strategies to restore damaged inflammatory ion transport and improve endo/epithelial barrier function
- Translational Research
  - Analysis of the anti-inflammatory, pro-resolution and alveolar repair mediating capacity of mesenchymal stem cells
  - Characterization of pulmonary dendritic cell (DC) subsets in the pneumococcal pneumonia mouse model
  - Purification and molecular genetic characterization of DC subsets with respect to inflammatory candidate genes relevant to pulmonary barrier dysfunction in the course of pneumococcal pneumonia
  - Review of the pathogenetic relevance of identified candidate molecules by knockout and inhibitor experiments in the S. pneumoniae-induced lung injury mouse model
- Clinical Research
  - Perform a dose-escalating pilot study with chemically defined lipid infusions in critically ill patients (NCTC1146821, EudraCT 2010-021018-49)

Goal 4 – Preventive Strategies

- Establish a pneumococcal colonisation/invasion model in the mouse with normal versus impaired mucociliary clearance (ENaC tg)
- Evaluation of pneumococcal protein-based immunization in pneumococcal colonization invasion model

2013 Research Highlights – Pneumonia and Acute Lung Injury

Research Highlight #1

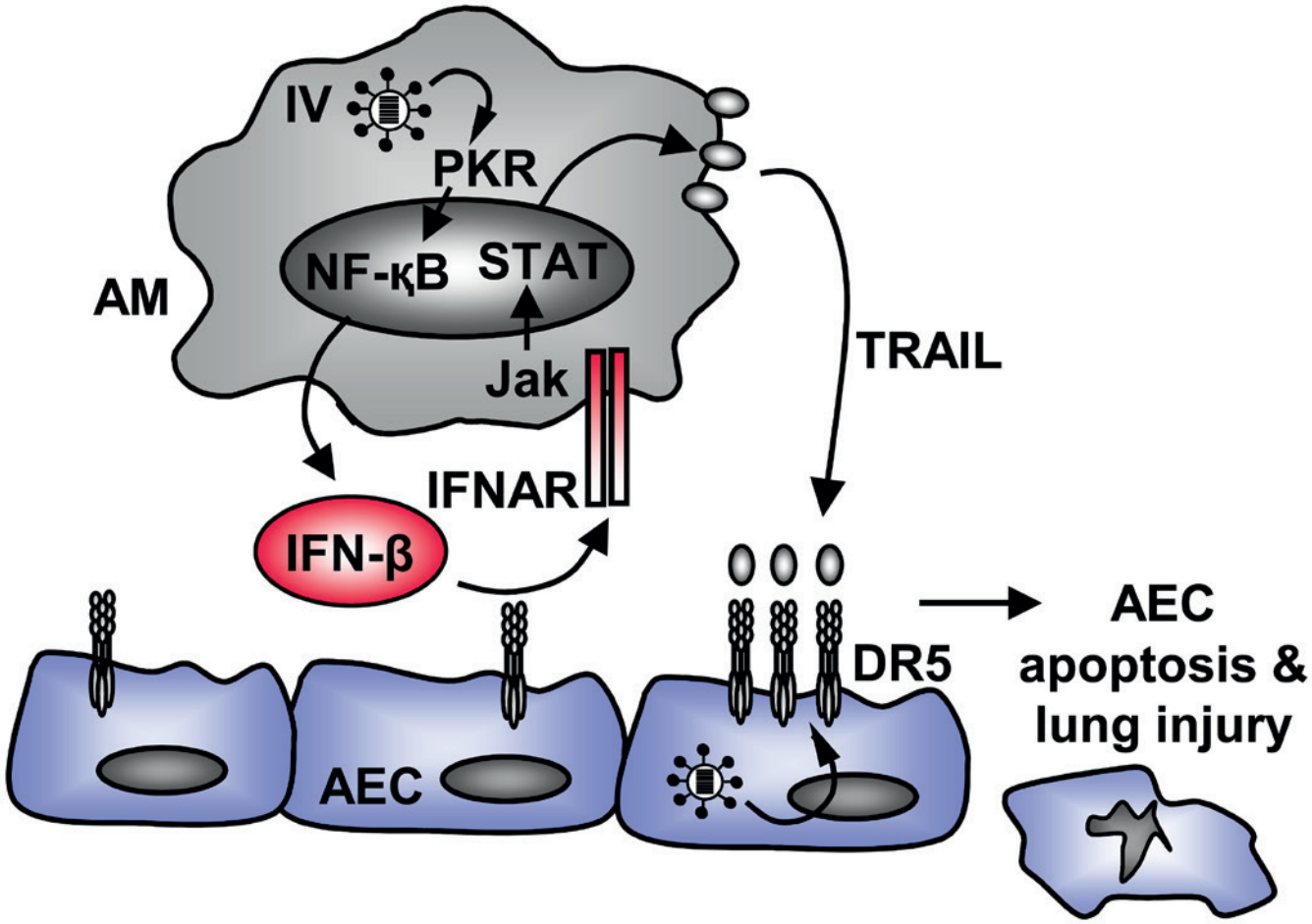


Figure 1. Macrophage-expressed IFN-β mediates apoptotic alveolar epithelial injury in severe influenza virus pneumonia. *The figure shows a model of IFN-β mediated pro-apoptotic AM-AEC cross-talk in IV-induced lung injury. IFN-β is released from IV-infected AM in a PKR- and NF-κB-dependent way and induces expression and release of macrophage TRAIL via autocrine IFNAR activation. Macrophage TRAIL induces AEC apoptosis via its receptor DR5 which is constitutively expressed on non-infected and upregulated on IV-infected AEC. Through this signaling cascade, IFN-β significantly contributes to AEC damage and lung injury during severe IV pneumonia. (AEC – alveolar epithelial cell; AM – alveolar macrophage; IFNAR - interferon-α/β receptor; IFN- β – interferon β; IV – influenza virus; JAK – Janus Kinase; PKR – Protein Kinase R; STAT - Signal Transducer and Activator of Transcription; TRAIL – TNF-related apoptosis inducing ligand) Figure from Högner K, Wolff T, Pleschka S, Plog S, Gruber AD, et al. (2013) Macrophage-expressed IFN-β Contributes to Apoptotic Alveolar Epithelial Cell Injury in Severe Influenza Virus Pneumonia. PLoS Pathog 9(2): e1003188. doi:10.1371/journal.ppat.1003188*

Research Highlight #2

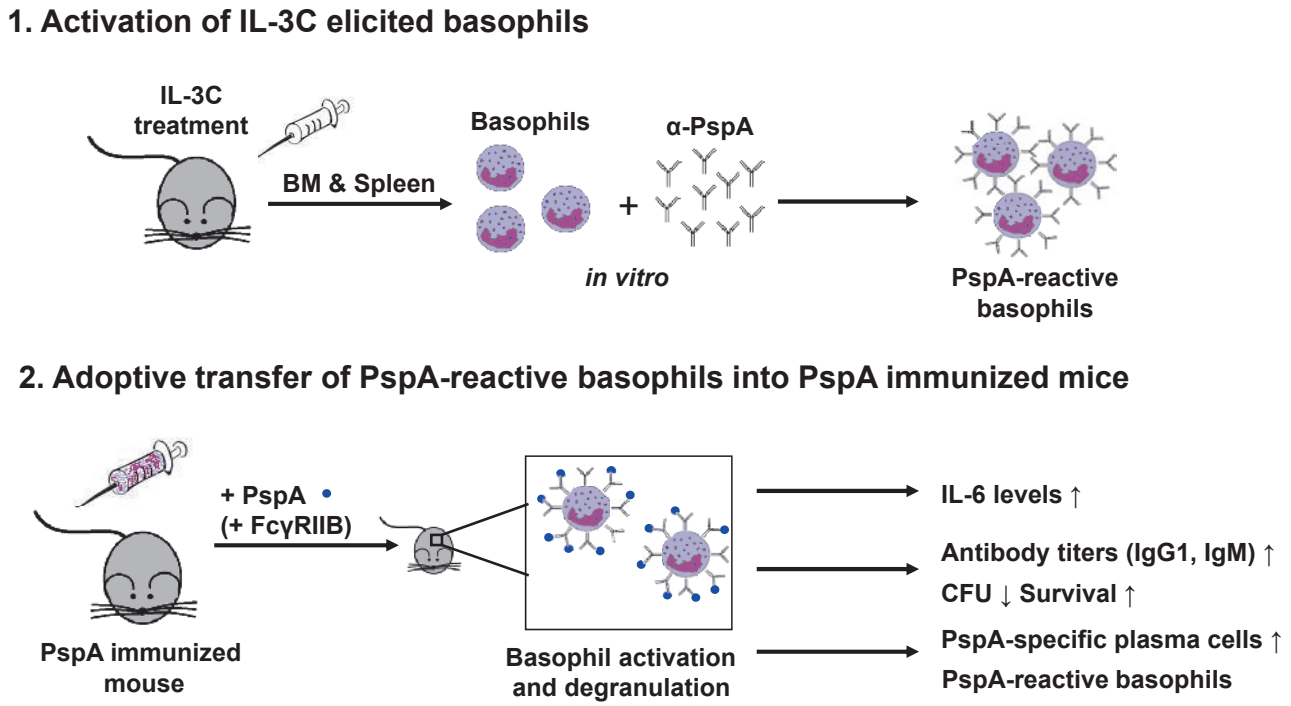


Figure 2. Basophil expansion protects against invasive pneumococcal disease in mice. *Mice treated with IL-3, which increased basophil pool sizes, and mice receiving a single basophil transfusion responded with significantly higher pneumococcal surface protein A (PspA)-specific antibody titers after immunization with PspA. Importantly, however, just a single transfusion of flow-sorted basophils into mice before secondary immunization with PspA significantly protected mice from lethal invasive pneumococcal disease (IPD). Moreover, concomitant blockade of inhibitory FcγRIIB on transfused basophils further substantially increased basophil-mediated protection against IPD in mice. This study is the first to find that a single transfusion of basophils is sufficient to boost proteinbased memory responses against pneumococcal protein antigens, thereby providing significant protection against IPD in mice. (Bischof A, Brumshagen C, Ding N, Korchhof G, Briles DE, Gessner JE, Welte T, Mack M, Maus UA. Basophil expansion protects against invasive penumococcal disease in mice.] Infect Dis, 2014 epub ahead of print)*

Research Highlight #3

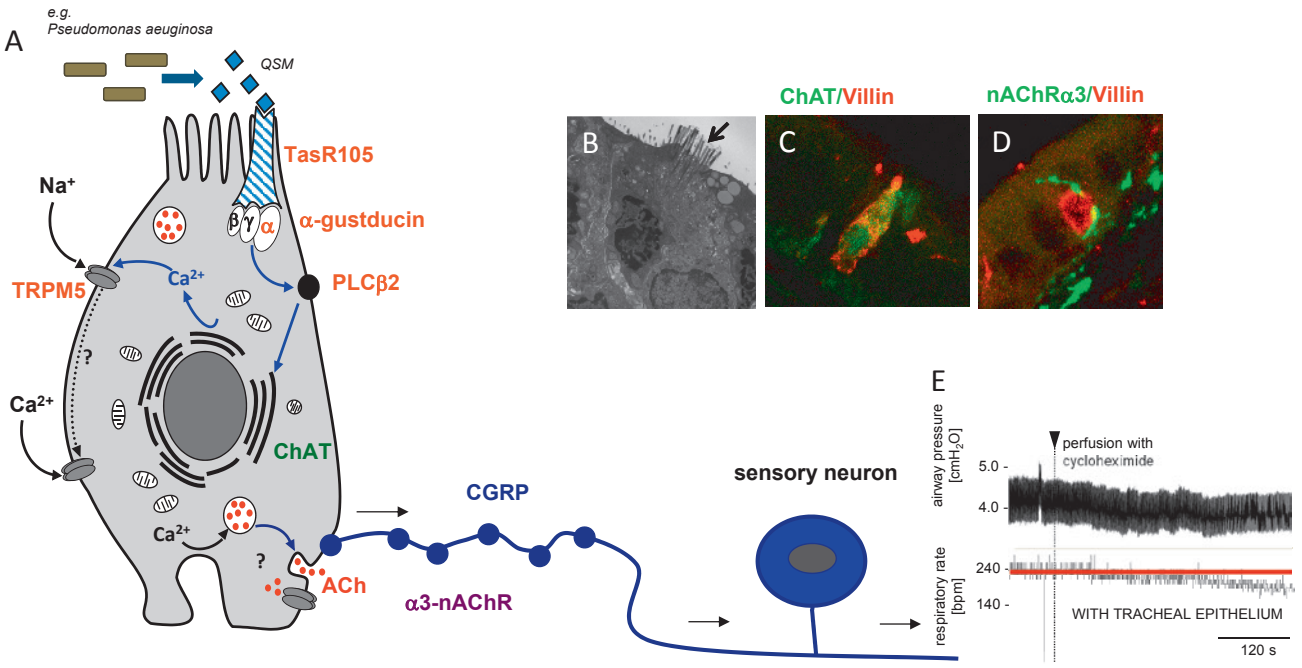


Figure 3. Specialized airway epithelial cells serve as sentinels of the airways by detecting hazardous luminal content. *Because of their typical brush of microvilli (B, arrow) containing the structural protein villin (C, D) these cells are called "brush cells". They carry canonical taste receptors (TasR) and the canonical taste transduction cascade members alpha (a)-Gustducin, phospholipase C beta 2 (PLCb2) and transient receptor potential ion channel member 5 (TRPM5). Brush cells produce the messenger molecule acetylcholine (ACh) via the enzyme choline acetyltransferase (ChAT). Upon stimulation with bacterial bitter tasting substances such as cycloheximide and quorum sensing molecules (QMS) ACh is released (A, red circles). This stimulation leads to excitation of peptidergic (CGRP, calcitonin gene-related peptide, blue circles in A) nerve terminals from sensory neurons carrying nicotinic ACh receptors (nAChRa3, C; nerve fibers in blue in A) and innervating brush cells (D). Thereby, they initiate protective respiratory reflexes such as drop in respiratory rate that prevents from further inhalation of the substances into the lower respiratory tract (E).*

Modified from Krasteva et al., Proc Natl Acad Sci USA 2011; Krasteva et al., Life Sci 2012; Krasteva and Kummer, Histochem Cell Biol, 2012; Kummer and Krasteva-Christ, Curr Opin Pharmacol, 2014.

Research Highlight #4

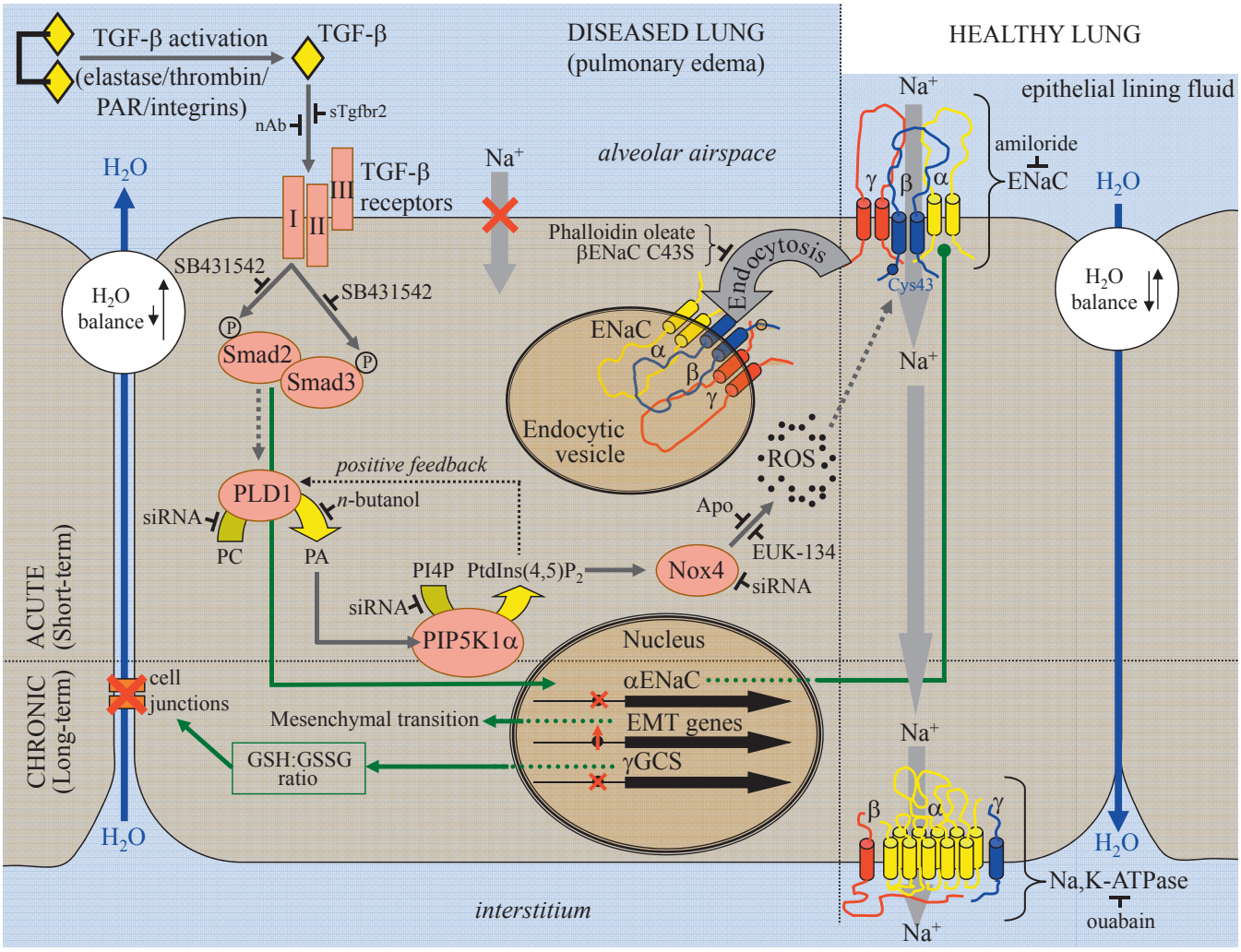


Figure 4. TGF-ß directs ENaC trafficking: implications for disturbed alveolar ion and fluid transport in acute lung injury. *In healthy lungs, latent TGF-β is inactive, and the Na+/K+-ATPase and ENaC drive Na+ absorption, maintaining fluid influx and reabsorption in equilibrium, and epithelial lining fluid volume at an appropriate level. After activation of latent TGF-β by elastase-, integrin- or protease-activated receptor (PAR)-dependent mechanisms in acute lung injury an acute effect of TGF-β on alveolar fluid reabsorption was observed. In the unique signaling pathway delineated (dark gray arrows), TGF-β, acting through the type I TGF-β receptor, induces Smad2/3 phosphorylation, which activates PLD1, which generates PA from phosphatidylcholine (PC). PA activates PIP5K1α, which drives NOX4 activation, perhaps by PtdIns(4,5)P2 formation from phosphatidylinositol 4′-monophosphate (PI4P). Activated NOX4 generates ROS, which promote βENaC internalization that is dependent on Cys43, which leads to loss of Na+-absorbing capacity of the epithelial cell and alveolar flooding, promoting persistence of alveolar edema. (Peters DM, Vadasz I, Wujak L, Wygrecka M, Olschewski A, Becker C, Herold S, Papp R, Mayer K, Rummel S, Brandes RP, Günther A, Waldegger S, Eickelberg O, Seeger W, Morty RE. TGF-ß directs ENaC trafficking: implications for disturbed alveolar ion and fluid transport in acute lung injury. Proc Natl Acad Sci U S A 2014; 111(3):E374-83)*

Number of papers published by DZL Faculty in 2013 - Disease Area Pneumonia and Acute Lung Injury: 35

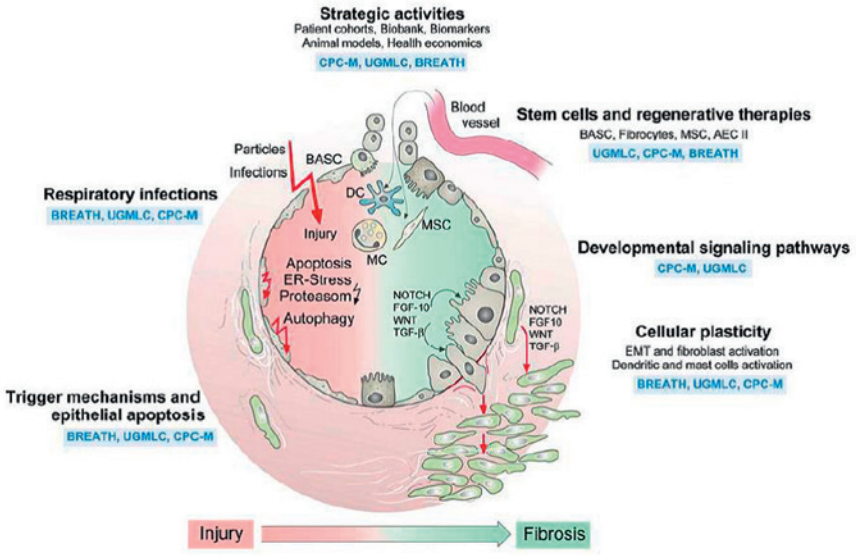
Highlighted Publications

1. Bischof A, Brumshagen C, Ding N, Kirchhof G, Briles DE, Gessner JE, Welte T, Mack M, Maus UA. Basophil expansion protects against invasive pneumococcal disease in mice. J Infect Dis 2014.
2. Herold S, Hoegner K, Vadasz I, Gessler T, Wilhelm J, Mayer K, Morty RE, Walmrath HD, Seeger W, Lohmeyer J. Inhaled granulocyte/macrophage colony-stimulating factor as treatment of pneumonia-associated acute respiratory distress syndrome. American Journal of Respiratory and Critical Care Medicine 2014;189:609-611.
3. Hogner K, Wolff T, Pleschka S, Plog S, Gruber AD, Kalinke U, Walmrath HD, Bodner J, Gattenlohner S, Lewe-Schlosser P, Matrosovich M, Seeger W, Lohmeyer J, Herold S. Macrophage-expressed ifn-beta contributes to apoptotic alveolar epithelial cell injury in severe influenza virus pneumonia. PLoS Pathogens 2013;9:e1003188.
4. Peters DM, Vadasz I, Wujak L, Wygrecka M, Olschewski A, Becker C, Herold S, Papp R, Mayer K, Rummel S, Brandes RP, Gunther A, Waldegger S, Eickelberg O, Seeger W, Morty RE. Tgf-beta directs trafficking of the epithelial sodium channel enac which has implications for ion and fluid transport in acute lung injury. Proceedings of the National Academy of Sciences of the United States of America 2014;111:E374-383.
5. Rotta Detto Loria J, Rohmann K, Droemann D, Kujath P, Rupp J, Goldmann T, Dalhoff K. Haemophilus influenzae infection upregulates the nlrp3 inflammasome and leads to caspase-1-dependent secretion of interleukin-1beta - a possible pathway of exacerbations in copd. PloS One 2013;8:e66818.

Diffuse Parenchymal Lung Disease (DPLD)

Disease Area Leaders	Prof. Dr. Oliver Eickelberg (CPC-M) Prof. Dr. Andreas Günther (UGMLC)
Participating DZL Partner Sites	BREATH, CPC-M, TLRC, UGMLC
Number of Participating DZL Faculty	28

Diffuse parenchymal lung diseases (DPLD) comprise more than 100 different entities yet share similar pathomechanistic principles, including progressive fibrosis of the pulmonary interstitium, distortion of normal lung architecture, and respiratory failure. Fibrotic alterations in DPLD can occur secondary to acute or chronic lung injury provoked by chemotherapy, toxin inhalation, collagen vascular disease, ventilation, or as an idiopathic entity (idiopathic interstitial pneumonia). Most DPLD patients exhibit a poor prognosis in the absence of medical treatment. One form of DPLD, Idiopathic Pulmonary Fibrosis (IPF), in particular, displays a progressive, devastating, and ultimately fatal course of disease which is largely resistant to medical treatment. As such, lung transplantation remains the only therapeutic intervention with a known survival benefit for IPF patients. Due to the urgent unmet medical need, the DZL DPLD program primarily focuses on IPF. The DZL aims to identify novel molecular paradigms and targets for the treatment of IPF, with the expectation that such discoveries will be transferable to positive outcomes for patients with other forms of DPLD.



(AEC II – alveolar type II cells ; BASC – bronchioalveolar stem cells; DC – dendritic cell; FGF – Fibroblast Growth Factor; MC – mast cell; MSC – mesenchymal stem cell; TGF – transforming growth factor)

Depicted above are the key goals of the Disease Area DPLD. In addition to strategic activities aiming to establish novel models of lung fibrosis and to facilitate access to human fibrotic tissues, the DZL disease area DPLD is actively performing research in the field of initial trigger mechanisms and relevant second hits such as respiratory infections. DZL scientists are also clarifying the role of the reactivation of developmental signaling pathways, investigating the importance of cellular plasticity for the development of the fibrotic tissue, and scrutinizing the therapeutic role of cell-based therapies in the field of DPLD.

Goals Followed in 2013 – DPLD

Goal 1 – Strategic Activities

- Creation of a DZL wide mutually shared patient registry
- Establishment of additional animal models for lung fibrosis and bronchopulmonary dysplasia (BPD)
- Evaluation of costs, health-related quality of life, and economic viability of new therapeutic approaches

Goal 2 – Trigger Mechanisms of DPLD and Epithelial Apoptosis

- The role of ER stress signaling pathways in lung fibrosis
- The role of proteasome function for ER-stress induced apoptosis in IPF
- Elucidation of the subcellular distribution and binding partners of Hermansky-Pudlak Syndrome gene products
- Defective lysosomal transport and autophagy in lung fibrosis

Goal 3 – Developmental Signaling Pathways in DPLD

- Preparation and analysis of transgenic animal models of epithelial cell-lineage tracing
- Evaluation and standardization of Wnt-inducible signaling protein-1 bioassays as a diagnostic biomarker for DPLD
- Identification of critical cell type-specific components of the FGF, Wnt and Notch signaling in DPLD

Goal 4 – Cellular Plasticity and Crosstalk in DPLD

- Description of the timing and pathological relevance of epithelial-mesenchymal transition in IPF
- Identification of key molecules in the remodeling of extracellular matrix in IPD and BPD
- Definition of an immune cell-mediated therapeutic approach for attenuating pulmonary fibrosis in animal models
- Evaluation of appropriate indicators/variables that allow early diagnosis of changes in the lungs (to prevent the development of BPD)

Goal 5 – Respiratory Infections in Lung Fibrosis

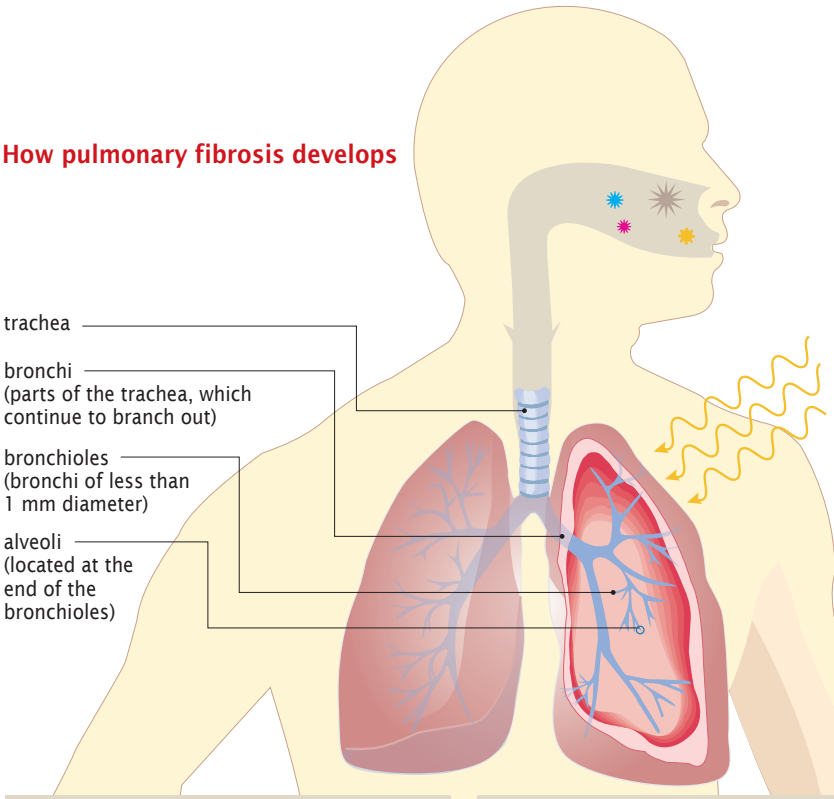
- The impact of Gram +/Gram - bacteria on onset and progression of pulmonary fibrosis
- Elucidation of the influence of pulmonary fibrosis on the clearance of pathogens from the lungs
- Description of the microbiomes of IPF patients

Goal 6 – Stem/Progenitor Cells and Regenerative Therapies in DPLD

- Characterization of the distribution and function of broncho-alveolar stem cells
- Evaluation of the suitability of fibrocytes as predictive biomarkers in DPLD
- Identification and characterization of appropriate cell populations offering for “stem cell treatment”; assessment of optimal application strategies

2013 Research Highlights – DPLD

Research Highlight #1



Causes

- harmful substances in the air (e.g. asbestos, silica dust, mold, toxic gases and fumes)
- recurrent and chronic infection
- systemic disorders (e.g. sarcoidosis)
- medication, radiation (e.g. cancer toxins, radiation therapy)
- idiopathic pulmonary fibrosis (cause unknown)

Disease

A pathological remodeling process in the lung tissue causes proliferation of connective tissue. This leads to thickening and scarring of lung tissue. Breathing gradually becomes more difficult.

Consequences

- shortness of breath
- dry cough
- blue, cyanotic lips
- finger clubbing at the base of the nails
- powerlessness, fatigue and reduced performance

Source: AtemWeg – The Lung Disease Research Foundation

Clinical Developments in the Disease Area DPLD. DZL scientists and clinicians are involved in the initiation and conduct of clinical studies with the aim of developing more effective treatments for interstitial lung. In 2013 the results of two randomized, placebo-controlled studies were published. Those studies investigated the effectiveness of endothelin-receptor-antagonists in patients with mild to moderately severe idiopathic pulmonary fibrosis (IPF). In one study the effect of ambrisentan was investigated. All patients who were enrolled underwent right-heart-catheterization to diagnose (or exclude) pulmonary hypertension. In the enrolled population of mild to moderately severe IPF patients the prevalence of precapillary pulmonary hypertension was found to be 10 percent. The trial was stopped early for futility after a planned interim analysis. At that point there was a significant negative effect of ambrisentan on disease progression and in addition there were negative trends on hospitalization and death. As a consequence ambrisentan is contraindicated in IPF-patients with pulmonary hypertension. This finding is an important example illustrating that sometimes off-label use of targeted pulmonary hypertension treatments may potentially be harmful (Raghu G et al., Ann Intern Med 2013). Another study investigated the effect of macitentan, a dual endothelin-receptor antagonist, on disease progression in mild to moderately severe IPF patients. Primary endpoint was the decline of the forced vital capacity after one year. The trial was fully enrolled but showed no significant treatment effect. The MUSIC trial is the last of several randomized controlled trials that investigated the potential effect of endothelin-receptor antagonists on IPF patients. None of these trials showed a beneficial treatment effect (Raghu G. et al., Eur Respir J 2013); thus at least for IPF-patients diagnosed according to the current clinical standards, endothelin-receptor antagonists are not efficacious.

The investigation of the development and the progression of interstitial lung disease shows many achievement-shas and has direct impact, translating into classification and treatment guidelines for that heterogeneous group of diseases. In 2013 the international multidisciplinary consensus-classification of idiopathic interstitial pneumonias (originally published in 2002) was updated. The originally proposed seven different entities of idiopathic interstitial pneumonias were recategorized into three groups: chronic fibrotic disease (idiopathic pulmonary fibrosis and nonspecific interstitial pneumonia), smoking-related interstitial lung disease (respiratory bronchiolitis-interstitial lung disease and desquamative interstitial pneumonia) and acute or subacute interstitial pneumonia (cryptogenic organizing pneumonia and acute interstitial pneumonia). In addition, several very rare interstitial lung diseases were described, some of them for the first time, as for example pleuro parenchymal fibroelastosis and bronchocentric interstitial pneumonia (Travis WD et al., Am J Respir Crit Care Med 2013). Furthermore the German treatment guideline document that is based on the international, evidence-based IPF guideline was updated in 2013. It summarizes the current diagnostic and therapeutic standards for IPF patients and adapts the international recommendations to the German health system, thereby integrating the most recent scientific literature (Behr J et al., Pneumologie 2013).

References

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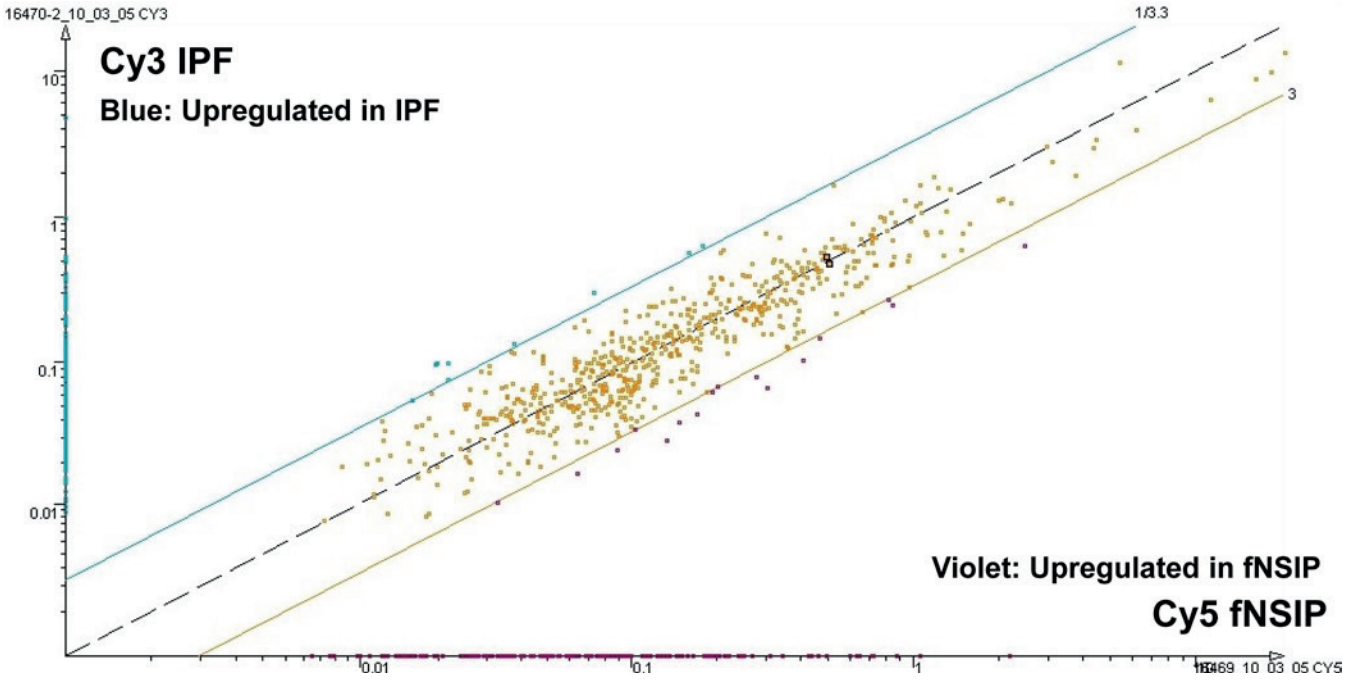
- Raghu G, Million-Rousseau R, Morganti A, Perchenet L, Behr J, Group MS. Macitentan for the treatment of idiopathic pulmonary fibrosis: the randomised controlled MUSIC trial. Eur Respir J. 2013 Dec;42(6):1622-32. PubMed PMID: 23682110.
- Travis WD, Costabel U, Hansell DM, King TE, Jr., Lynch DA, Nicholson AG, et al. An official American Thoracic Society/European Respiratory Society statement: Update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. Am J Respir Crit Care Med. 2013 Sep 15;188(6):733-48. PubMed PMID: 24032382.
- Behr J, Gunther A, Ammenwerth W, Bittmann I, Bonnet R, Buhl R, et al. [German guideline for diagnosis and management of idiopathic pulmonary fibrosis]. Pneumologie. 2013 Feb;67(2):81-111. PubMed PMID: 23325398. S2K-Leitlinie zur Diagnostik und Therapie der idiopathischen Lungenfibrose.

Research Highlight #2



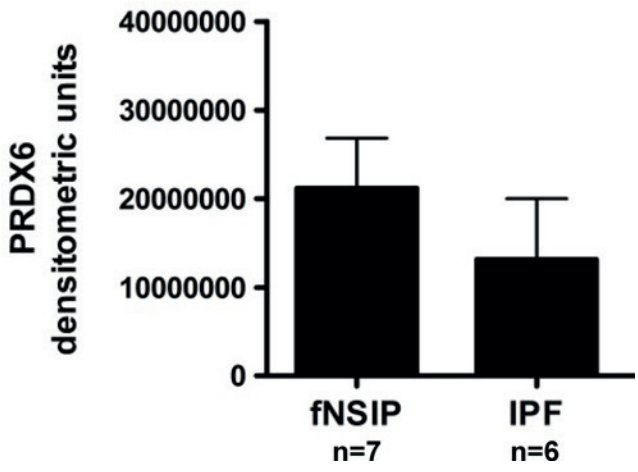
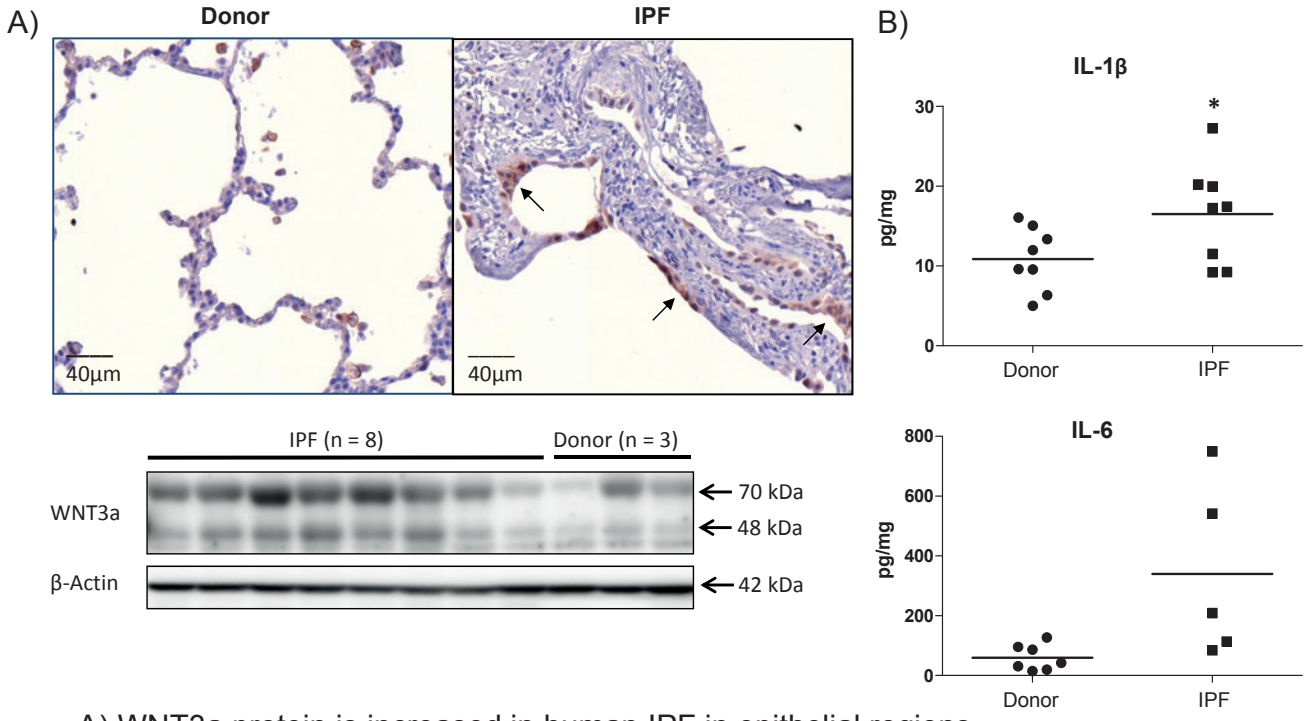


Figure 2. Differential diagnosis of patients with IPF and non-specific interstitial pneumonia. *A differential diagnosis of patients with IPF and non-specific interstitial pneumonia (NSIP) is possible according to current international guidelines which have been elaborated with contributions from DZL scientists. Clinical, radiological, and histopathological appearance and prognosis of the disease are taken into consideration to assign the patients to the corresponding disease. However, in cases with familial background (approx. 15%) both IPF as well as NISP specific characters are found. Transition from NSIP to IPF appears in sporadic IPF. Moreover in IPF lungs areas with NSIP typical histopathological morphology are visible suggesting pathomechanistic similarities between fibrotic NSIP (fNSIP) and IPF. Therefore, DZL scientists analyzed the proteome of both NSIP and IPF patients by differential in gel electrophoresis (DIGE) in order to identify similarities and differences in protein regulation in the lung of patients with both diseases. Indeed as shown in the figure above protein regulation in NSIP and IPF is comparable (all dots between both solid lines). Only few proteins were differentially regulated. One of them is the antioxidant Perioxiredoxin (Prdx6). Prdx6 is expressed mainly in alveolar Type II cells and has been shown to exert protective function on the alveolar epithelium. Thus, the differences concerning survival and prognosis of fNSIP and IPF are possibly due to a slightly improved epithelial protection in fNSIP. (Korfei M, Henneke I, Markart P, von der Beck D, Ruppert C, Mahavadi P, Klepetko W, Fink L, Meiners S, Krämer O, Seeger W, Vancheri C, Günther A. Comparative Proteome Analysis of Lung Tissue from Patients with Idiopathic Pulmonary Fibrosis (IPF), Non-specific Interstitial Pneumonia (NSIP) and Organ Donors. J Proteomics 85:109-28; 2013.)*

Research Highlight #3



A) WNT3a protein is increased in human IPF in epithelial regions and in whole lung homogenate

B) IL-1 and IL-6 level is increased in patient BALF

Figure 3. WNT/β-Catenin Signaling Induces Interleukin 1β Expression by Alveolar Epithelial Cells in Pulmonary Fibrosis. *Recently, reactivation of the developmental WNT/β-catenin pathway has been linked with pulmonary fibrosis. The cell-specific mechanisms and mediators of WNT/β-catenin signaling in the lung, however, remain elusive. In this investigation an unbiased gene expression screen was applied to identify epithelial cell-specific mediators of WNT/β-catenin signaling. It was found that proinflammatory cytokine interleukin (IL) 1β is one of the most upregulated genes in primary murine alveolar epithelial type II (ATII) cells after WNT3a treatment. Importantly, primary fibrotic ATII cells isolated from lungs subjected to bleomycin secreted enhanced IL-1β and IL-6 in vitro. Furthermore, local application of recombinant WNT protein in Wnt reporter animals led to WNT/β-catenin activation in epithelial cells along with a significant increase in IL-1β and IL-6 in vivo. Finally, increased WNT3a protein in fibrotic alveolar epithelium accompanied by enhanced IL-1β and IL-6 level in BALF from patients with idiopathic pulmonary fibrosis has been found. Taken together, these findings revealed that the alveolar epithelium is a relevant source of proinflammatory cytokines induced by active WNT/β-catenin in pulmonary fibrosis. Thus, WNT/β-Catenin signaling represents a novel link between developmental pathway reactivation and inflammation in the development of pulmonary fibrosis. (Aumiller et al., WNT/β-catenin signaling induces IL-1β expression by alveolar epithelial cells in pulmonary fibrosis. Am J Respir Cell Mol Biol. 2013 Jul;49(1):96-104.)*

Number of papers published by DZL Faculty in 2013 – Disease Area DPLD: 29

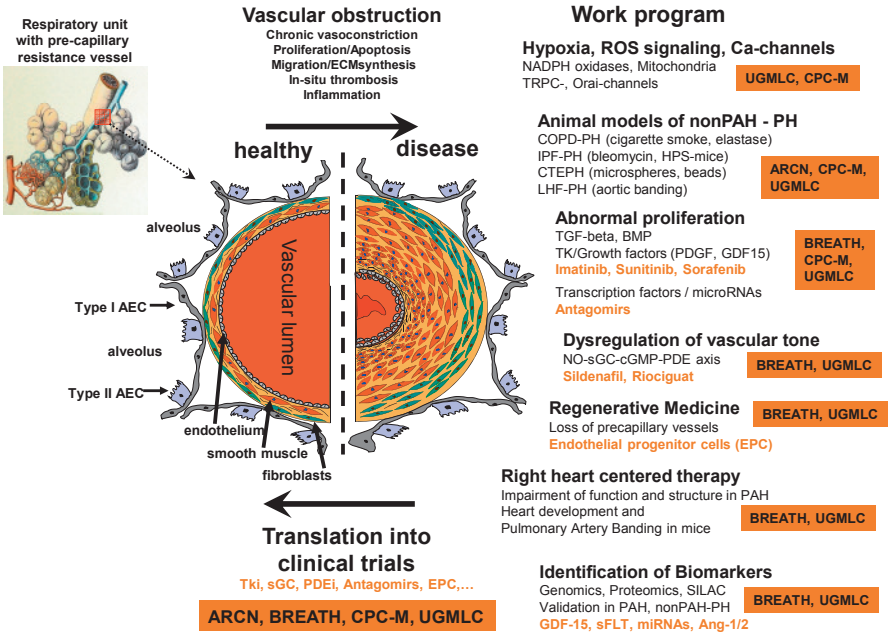
Highlighted Publications

1. Burgstaller G, Oehrle B, Koch I, Lindner M, Eickelberg O. Multiplex profiling of cellular invasion in 3d cell culture models. PloS One 2013;8:e63121.
2. Danopoulos S, Parsa S, Al Alam D, Tabatabai R, Baptista S, Tiozzo C, Carraro G, Wheeler M, Barreto G, Braun T, Li X, Hajihosseini MK, Bellusci S. Transient inhibition of fgfr2b-ligands signaling leads to irreversible loss of cellular beta-catenin organization and signaling in aer during mouse limb development. PloS One 2013;8:e76248.
3. Huppmann P, Sczepanski B, Boensch M, Winterkamp S, Schonheit-Kenn U, Neurohr C, Behr J, Kenn K. Effects of inpatient pulmonary rehabilitation in patients with interstitial lung disease. The European Respiratory Journal 2013;42:444-453.
4. Kaltenborn E, Kern S, Frixel S, Fragnet L, Conzelmann KK, Zarbock R, Griese M. Respiratory syncytial virus potentiates abca3 mutation-induced loss of lung epithelial cell differentiation. Human Molecular Genetics 2012;21:2793-2806.
5. Nkyimbeng T, Ruppert C, Shiomi T, Dahal B, Lang G, Seeger W, Okada Y, D'Armiento J, Gunther A. Pivotal role of matrix metalloproteinase 13 in extracellular matrix turnover in idiopathic pulmonary fibrosis. PloS One 2013;8:e73279.

Pulmonary Hypertension

Disease Area Leaders	Prof. Dr. Hossein Ardeschir Ghofrani (UGMLC) Prof. Dr. Marius Höper (BREATH)
Participating DZL Partner Sites	ARCN, BREATH, CPC-M, TLRC, UGMLC
Number of Participating DZL Faculty	26

Pulmonary hypertension (PH) is a disease of the pulmonary vasculature, which leads to shortness of breath, dizziness, fainting, and ultimately right heart failure. Five PH subclasses have been defined and all variants of PH together are estimated to affect up to 100 million people worldwide. The vascular pathology of PH is characterized by pulmonary vasoconstriction and by abnormal ("pseudo-malignant") remodeling processes of all vessel layers. Vascular smooth muscle cell (SMC) proliferation is a prominent feature in virtually all PH entities. These remodeling processes result in severe loss of cross-sectional area, vascular pruning, and a concomitant increase in right ventricular afterload. Current PH therapy provides symptomatic relief and improves prognosis, but falls short as to reestablishment of structural and functional lung vascular integrity as a basis for handicapped-free long-term survival. The restoration of physiological vascular structure and function (reverse remodeling) represents the major therapeutic goal of the DZL PH team.



Vascular Remodelling and Reverse Remodelling in Pulmonary Hypertension. *Putative therapeutic targets are indicated. (Acronyms: NO, nitric oxide; sGC, soluble guanylate cyclase; cGMP, cyclic guanosine monophosphate; PDE, phosphodiesterase; TGF, (transforming growth factor)-b; BMP, bone morphogenetic protein; TK, tyrosine kinase; PDGF, platelet-derived growth factor; EGF, epidermal growth factor; VEGF, vascular endothelial growth factor; EPC, endothelial progenitor cells; TRPC, transient receptor potential cation channels; NADPH, nicotinamide adenine dinucleotide phosphate; TKi, tyrosine kinase inhibitor; AEC, alveolar epithelial cells.)*

Goals Followed in 2013 – Pulmonary Hypertension

Goal 1 - Basic Research in Pulmonary Hypertension – From Disease Genes To New Therapeutic Approaches

- Hypoxia, ROS signaling pathways and hypoxia-induced gene regulation in PH
  - Generation of transgenic mice with reactive oxygen species (ROS) sensitive fluorescent proteins
  - Detection of ROS in isolated lungs and isolated smooth muscle cells before and after hypoxia
  - Investigation of the mitochondrial respiratory chain and membrane potential and investigation of inhibitors
  - Examination of the role of HIF by the use of transgenic mice (prolyl hydroxylase (PHD) and Siah ubiquitin ligase)
- New calcium (Ca2 +) influx pathways in pulmonary hypertension and vascular dysfunction
  - Investigation of the pathophysiological role of the TRP and the store-operated Orai channels
  - Investigation of calcium signaling pathways using patch-clamp and single-cell fluorescence imaging in combination with functional studies on endothelial cells and smooth muscle cells
- Animal models for non-PAH PH
  - Establishment of the transaortic banding model (TAC) to study PH due to left ventricular disease; testing of new substances and those already approved for PAH treatment
  - Testing of new and approved compounds for treatment of PAH in animal models of DPLD
  - Establishment of a model for CTEPH (pulmonary embolism by injection of microparticles) to study PH, and for testing of new and approved compounds for treatment of PAH

Goal 2 – Translational Pulmonary Hypertension Research

- Promotion of vascular remodeling in PH: transcription factors and receptor tyrosine kinases
  - Study of the expression profiles of various growth factors in experimental and clinical PH and non-PAH PH
  - Examination of the profile of expression of receptor tyrosine kinases in human and experimental PAH tissue
  - Identification of growth factor receptors as potential biomarkers for monitoring treatment in human circulating monocytes
- Reverse remodeling by NO-guanylate cyclase-phosphodiesterase-axis
  - Investigation of sGC expression and activity of the various subunits and the signal connected molecules in experimental and clinical PH and non-PAH PH
  - Development of inhaled therapy strategies (e.g., nanoparticles)
  - Examination of the role of various PDE isoforms and their possible therapeutic potential for non-PAH PH (experimental and clinical)
- MicroRNAs and Antagomirs for the treatment of PH
  - Assessment of cell- and compartment-specific miRNA profiles in experimental PAH and non-PAH-PH models, and in human PAH tissue

    - Identification of promising drug targets and testing their antiproliferative capacity by antagomir treatment in vitro and in preclinical animal models
    - Identification of circulating miRNAs as potential biomarkers for the assessment of disease severity and treatment success
- Endothelial progenitor cell (EPC)-based revascularization of the lung
    - Conduct investigations to increase the pro-angiogenic potential of EPCs by prestimulation with homing-promoting factors
    - Isolation of EPCs in from human peripheral blood; manipulation of these cells by pharmacological approaches and transfection technology
- Treatment of PH with a focus on the right heart
    - Analysis of the expression and the functional role of genes regulated in right heart failure in response to increased afterload
    - Investigation of the effect of compounds approved for PAH on right ventricular function and structure in the pulmonary arterial banding model

Goal 3 – Clinical Pulmonary Hypertension Research

- Non-hypothesis-based screen for new biomarkers
    - Examination of tissue from patients with PAH or non-PAH PH compared to healthy individuals
    - Implementation of broad genome, transcriptome and epigenomanalyse screens in lung tissue and in selected compartments of the lung
    - Identification of potential biomarkers for the assessment of pulmonary vascular resistance and the load of the right ventricle in CTEPH patients
- Phenotyping of different PH entities and correlation with biomarker candidates
    - Identification of potential biomarkers for the assessment of disease severity and treatment success; differentiation of the various PH subtypes
- Early clinical studies
    - Conducting studies of sildenafil in ILD-PH: Long-term treatment (3 months) of ILD-PH patients with sildenafil

Research Highlight #1

Riociguat as a therapeutic option in chronic thromboembolic pulmonary hypertension – results of the CHEST-1 study. In the international, multicenter, randomized, placebo-controlled clinical CHEST-1 (Chronic Thromboembolic Pulmonary Hypertension Soluble Guanylate Cyclase-Stimulator Trial 1) trial, the efficacy and side-effect profile of the soluble guanylate cyclase stimulator riociguat in patients with chronic thromboembolic pulmonary hypertension (CTEPH) was investigated. To participate in the trial, patients had to be considered ineligible for surgery or to have persistent or recurrent pulmonary hypertension after pulmonary endarterectomy. A total of 261 patients underwent randomization and received at least one dose of study medication (173 patients in the riociguat group and 88 in the placebo group). At week 16, the 6-minute walk distance increased from baseline by a mean of 39 m in the riociguat group, as compared to a mean decrease of 6 m in the placebo group (least-squares mean difference, 46 m; 95% confidence interval [CI], 25 to 67; p<0.001). Significant improvements were also observed in the clinically relevant secondary end points, including pulmonary vascular resistance, NT-proBNP level, and WHO functional class. The US Food and Drug Administration (FDA) approved riociguat for the treatment of CTEPH in October 2013 in USA and the European Medicines Agency (EMA) in March 2014 in Europe.

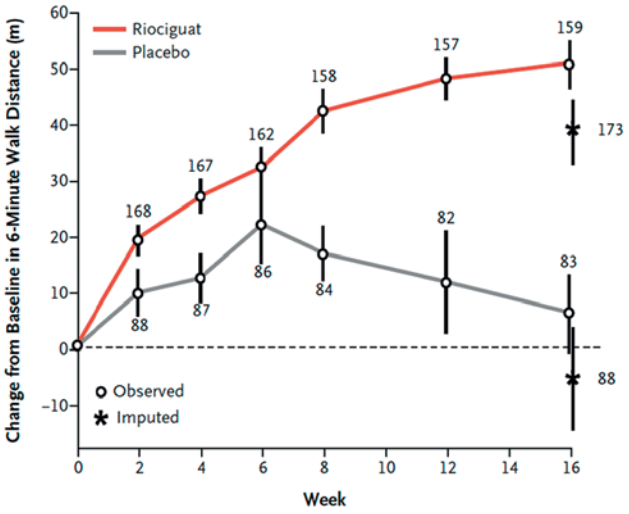


Figure 1. Mean Change from Baseline in the 6-Minute Walk Distance. *Mean changes from baseline in the distance walked in 6 minutes during the 16-week study are shown in the modified intention-to-treat population without imputation of missing values, with the imputed values also provided at week 16. The number at each data point indicates the number of patients included in the assessment at that time point. The least-squares mean difference in the distances at week 16 was 46 m (95% CI, 25 to 67; P<0.001). The last observed value (not including follow-up) was carried forward for patients who completed the study or withdrew; the worst value (0 m) was imputed in the case of death or clinical worsening without a termination visit or without a measurement at the termination visit. From New England Journal of Medicine, Ghofrani HA et al., Riociguat for the treatment of chronic thromboembolic pulmonary hypertension., Volume 369., Page 319-29. Copyright © 2013 Massachusetts Medical Society. Reprinted with permission.*

Research Highlight #2

Riociguat as a therapeutic option in pulmonary arterial hypertension – results of the PATENT-1 study. In the international, multicenter, randomized, placebo-controlled clinical PATENT-1 (Pulmonary Arterial Hypertension Soluble Guanylate Cyclase-Stimulator Trial 1) trial, the efficacy and side-effect profile of the soluble guanylate cyclase stimulator riociguat was investigated in patients with symptomatic pulmonary arterial hypertension (PAH). Patients with symptomatic PAH (idiopathic, familial, or associated with connective-tissue disease, congenital heart disease, portal hypertension with liver cirrhosis, or anorexigen or amphetamine use) were included if they had a pulmonary vascular resistance greater than 300 dyn sec cm−5, a mean pulmonary-artery pressure of at least 25 mm Hg, and a 6-minute walk distance of 150 to 450 m. A total of 443 patients were randomly assigned to receive placebo (126 patients), riociguat at individually adjusted doses up to 2.5 mg three times daily (254 patients), or riociguat at individually adjusted doses capped at 1.5 mg three times daily (63 patients). In this study, riociguat significantly improved exercise capacity in patients with PAH. This benefit was consistent in patients who were receiving endothelin-receptor antagonists or prostanoids and in those who were receiving no other treatment for the disease. Riociguat also significantly and consistently improved a range of secondary efficacy end points, including pulmonary hemodynamics, WHO functional class, and time to clinical worsening. Subsequently, the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) approved riociguat for the treatment of PAH in the USA (2013) and Europe (2014).

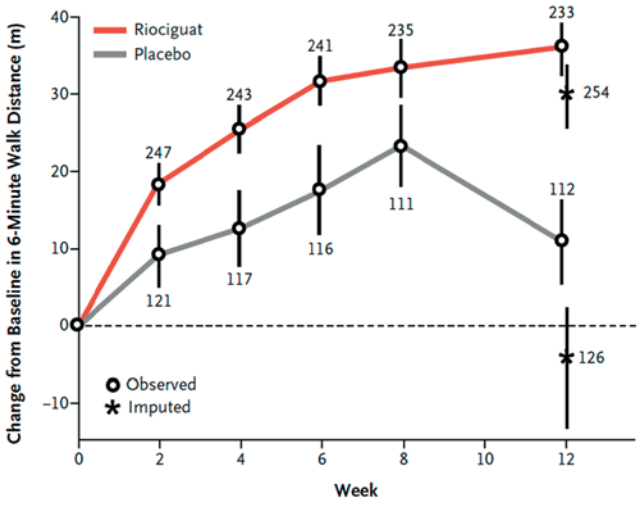


Figure 2. Mean Change from Baseline in the 6-Minute Walk Distance. *Mean changes from baseline in the distance walked in 6 minutes during the 12-week PATENT-1 study period are shown in the group that received riociguat at a dose up to 2.5 mg three times daily as compared with the placebo group. The data were analyzed in the modified intention-to-treat population without imputation of missing values; imputed values are provided at week 12. The number at each data point indicates the number of patients included in the assessment at that time point. The least-squares mean difference in the 6-minute walk distance at week 12 was 36 m (95% CI, 20 to 52; P<0.001). The last observed value (not including follow-up) was carried forward for patients who completed the study or withdrew; the worst value (0 m) was imputed in the case of death or clinical worsening without a termination visit or without a measurement at the termination visit. Ghofrani HA et al Riociguat for the treatment of pulmonary arterial hypertension., Volume 369., Page 330-40. Copyright © 2013 Massachusetts Medical Society. Reprinted with permission.*

Research Highlight #3

Classical Transient Receptor Potential Channel 1 in Hypoxia-induced Pulmonary Hypertension. Exposure to chronic hypoxia leads to the development of pulmonary hypertension (PH). This severe disease is characterized by vascular remodeling due to enhanced pulmonary arterial smooth muscle cell (PASMC) proliferation. The present study aimed to identify the role of transient receptor potential channel 1 (TRPC1) in chronic hypoxia-induced PH. TRPC1 shows hypoxia-dependent upregulation in PASMC. Furthermore, TRPC1 silencing or lacking causes impaired proliferation rate in response to chronic hypoxia. Additionally, TRPC1 deficient mice possess less vascular remodeling, characterized by less vascular muscularization of small vessels. Moreover, TRPC1 deficient mice revealed reduced right ventricular systemic pressure in response to chronic hypoxia. Our results suggest a significant role of TRPC1 in pulmonary vascular remodeling underlying PH pathogenesis.

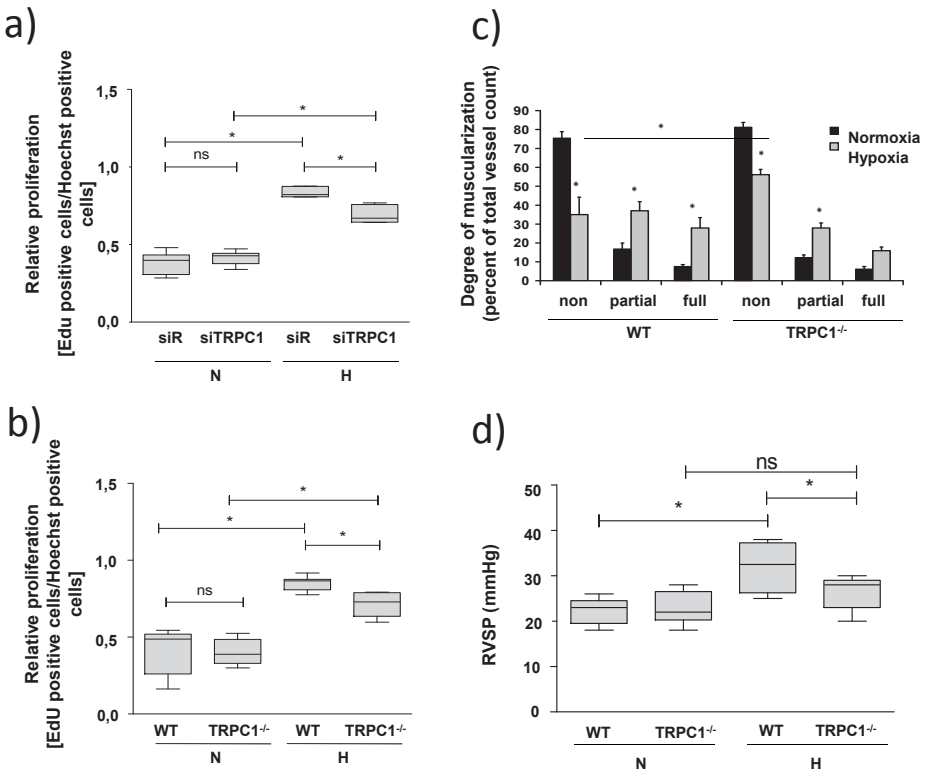


Figure 3. a) and b) *Hypoxia (H, 24h, 1% O2) -induced proliferation of (a) murine PASMC after knockdown of TRPC1 expression (siTRPC1) compared to control (siR) (n=4-5) and (b) murine PASMC isolated from WT and TRPC1 -/- (n=5-7) assessed by cell counting using 5-ethynyl-uridine (EdU) based proliferation assay.* ***c)*** *Degree of muscularization in small pulmonary vessels (external diameter of 20-70µm) from WT and TRPC1 -/- mice exposed to normoxia (21d; 21% O2) or chronic hypoxia (21d; 10% O2). The proportion of fully muscularized (full), partially muscularized (partial) and non muscularized (non) vessels is given in percentage of total vessel count (n=5).* ***d)*** *Right ventricular systolic pressure (RVSP) in WT and TRPC1-/- mice kept under normoxic (N, 21d; 21% O2) or chronic hypoxic (H, 21d; 10% O2) conditions (n=7). Adapted with permission of the American Thoracic Society. Copyright © 2013 American Thoracic Society. Malczyk, et al., 2013. Classical transient receptor potential channel 1 in hypoxia-induced pulmonary hypertension. 188: 1451-59. Official Journal of the American Thoracic Society.*

Research Highlight #4

Imatinib PH – Results of the IMPRES study. In the multicentre, randomized, double-blind, placebo-controlled 24 week-trial IMPRES (Imatinib in Pulmonary Arterial Hypertension, a Randomized Efficacy Study), 202 patients were enrolled and randomized in a 1:1 ratio to Imatinib or placebo. Patients were required to be symptomatic on ≥ 2 PAH therapies, with a PVR ≥ 800 dyne•s•cm-5. Male and female patients (≥ 18 years) with WHO functional class II through IV were enrolled if they met the criteria for one of the following categories of group 1 PH: idiopathic or heritable PAH; PAH associated with connective tissue disease; PAH after ≥ 1 year repair of congenital systemic to pulmonary shunt; or PAH associated with anorexigens or other drugs. Imatinib starting dose was 200 mg once daily and the dose was increased to 400 mg once daily after 2 weeks, if the starting dose was tolerated. The dose could be reduced to 200 mg if the 400 mg dose was not tolerated.

Imatinib significantly improved 6 minute walking distance and N-terminal pro-B-type natriuretic peptide levels at week 24 compared with placebo. Patients receiving Imatinib had greater improvements in hemodynamics; mean pulmonary arterial pressure (mPAP), cardiac output, pulmonary vascular resistance (PVR), and right atrial pressure (RAP) improved compared with placebo. But discontinuation of study medication and serious AEs were common in the Imatinib group, and further studies are required to assess the risk-benefit profile of Imatinib in patients with advanced PAH.

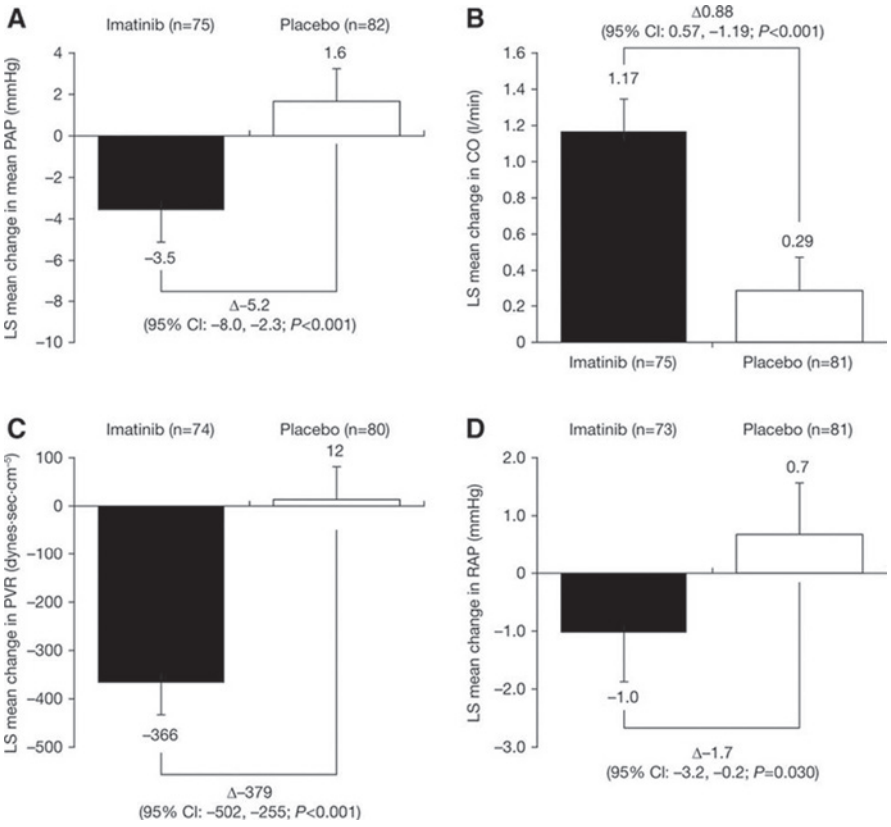


Figure 4. *Least squares (LS) mean changes from baseline to end of study in mean pulmonary artery pressure (PAP; A) cardiac output (CO; B), pulmonary vascular resistance (PVR; C), and right atrial pressure (RAP; D). Δ indicates LS mean difference between groups; and CI, confidence interval. Patients included in analyses of hemodynamic parameters include those who completed the study plus those who discontinued early but had a right heart catheterization performed at discontinuation. (Hoeper et al., Circulation 2013;127:1128-1138)*

Number of papers published by DZL Faculty in 2013 - Disease Area Pulmonary Hypertension: 56

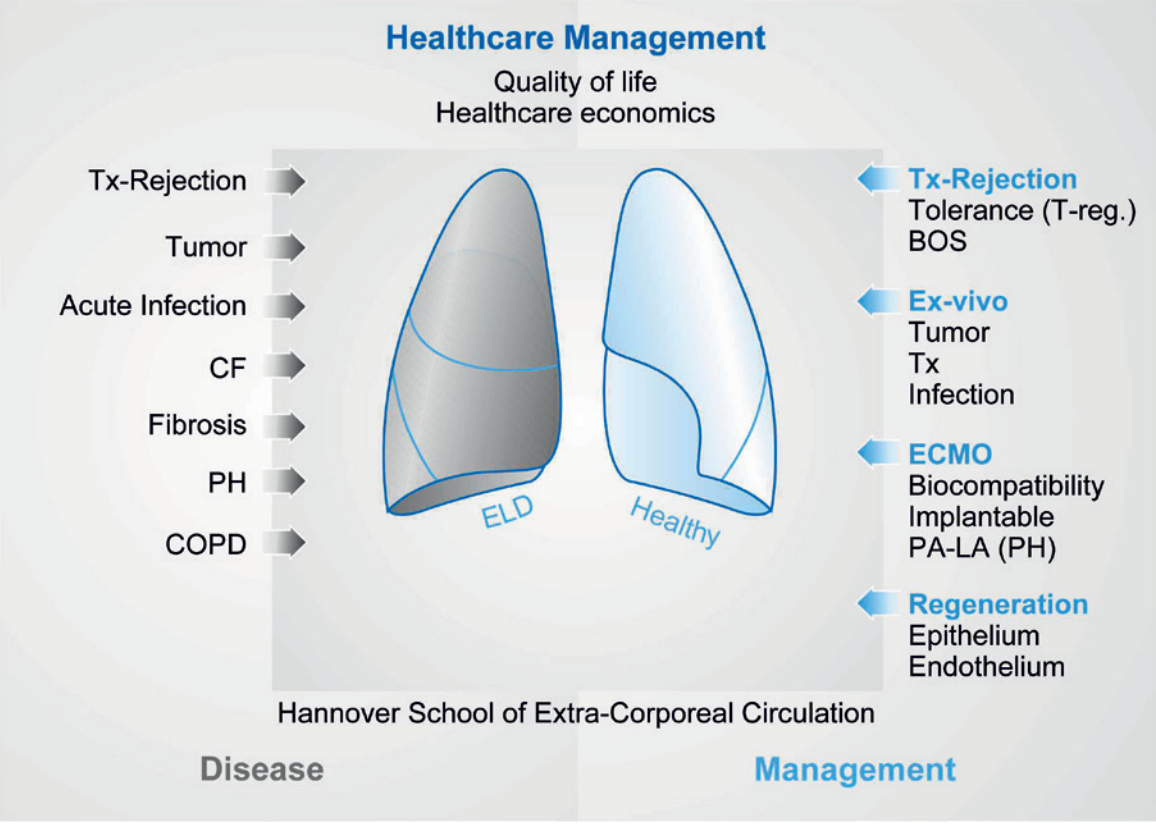
Highlighted Publications

1. Ghofrani HA, D'Armini AM, Grimminger F, Hoeper MM, Jansa P, Kim NH, Mayer E, Simonneau G, Wilkins MR, Fritsch A, Neuser D, Weimann G, Wang C, Group C-S. Riociguat for the treatment of chronic thromboembolic pulmonary hypertension. The New England Journal of Medicine 2013;369:319-329.
2. Ghofrani HA, Galie N, Grimminger F, Grunig E, Humbert M, Jing ZC, Keogh AM, Langleben D, Kilama MO, Fritsch A, Neuser D, Rubin LJ, Group P-S. Riociguat for the treatment of pulmonary arterial hypertension. The New England Journal of Medicine 2013;369:330-340.
3. Hoeper MM, Barst RJ, Bourge RC, Feldman J, Frost AE, Galie N, Gomez-Sanchez MA, Grimminger F, Grunig E, Hassoun PM, Morrell NW, Peacock AJ, Satoh T, Simonneau G, Tapson VF, Torres F, Lawrence D, Quinn DA, Ghofrani HA. Imatinib mesylate as add-on therapy for pulmonary arterial hypertension: Results of the randomized impres study. Circulation 2013;127:1128-1138.
4. Kojonazarov B, Sydykov A, Pullamsetti SS, Luitel H, Dahal BK, Kosanovic D, Tian X, Majewski M, Baumann C, Evans S, Phillips P, Fairman D, Davie N, Wayman C, Kilty I, Weissmann N, Grimminger F, Seeger W, Ghofrani HA, Schermuly RT. Effects of multikinase inhibitors on pressure overload-induced right ventricular remodeling. International Journal of Cardiology 2013;167:2630-2637.
5. Malczyk M, Veith C, Fuchs B, Hofmann K, Storch U, Schermuly RT, Witzenrath M, Ahlbrecht K, Fecher-Trost C, Flockerzi V, Ghofrani HA, Grimminger F, Seeger W, Gudermann T, Dietrich A, Weissmann N. Classical transient receptor potential channel 1 in hypoxia-induced pulmonary hypertension. American Journal of Respiratory and Critical Care Medicine 2013;188:1451-1459.
6. Olsson KM, Delcroix M, Ghofrani HA, Tiede H, Huscher D, Speich R, Grunig E, Staehler G, Rosenkranz S, Halank M, Held M, Lange TJ, Behr J, Klose H, Claussen M, Ewert R, Opitz CF, Vizza CD, Scelsi L, Vonk-Noordegraaf A, Kaemmerer H, Gibbs JS, Coghlan G, Pepke-Zaba J, Schulz U, Gorenflo M, Pittrow D, Hoeper MM. Anticoagulation and survival in pulmonary arterial hypertension: Results from the comparative, prospective registry of newly initiated therapies for pulmonary hypertension (compera). Circulation 2014;129:57-65.
7. Pak O, Sommer N, Hoeres T, Bakr A, Waisbrod S, Sydykov A, Haag D, Esfandiary A, Kojonazarov B, Veit F, Fuchs B, Weisel FC, Hecker M, Schermuly RT, Grimminger F, Ghofrani HA, Seeger W, Weissmann N. Mitochondrial hyperpolarization in pulmonary vascular remodeling. Mitochondrial uncoupling protein deficiency as disease model. American Journal of Respiratory Cell and Molecular Biology 2013;49:358-367.
8. Pulido T, Adzerikho I, Channick RN, Delcroix M, Galie N, Ghofrani HA, Jansa P, Jing ZC, Le Brun FO, Mehta S, Mittelholzer CM, Perchenet L, Sastry BK, Sitbon O, Souza R, Torbicki A, Zeng X, Rubin LJ, Simonneau G, Investigators S. Macitentan and morbidity and mortality in pulmonary arterial hypertension. The New England Journal of Medicine 2013;369:809-818.
9. Veith C, Schmitt S, Veit F, Dahal BK, Wilhelm J, Klepetko W, Marta G, Seeger W, Schermuly RT, Grimminger F, Ghofrani HA, Fink L, Weissmann N, Kwapiszewska G. Cofilin, a hypoxia-regulated protein in murine lungs identified by 2de: Role of the cytoskeletal protein cofilin in pulmonary hypertension. Proteomics 2013;13:75-88.

End-Stage Lung Disease

Disease Area Leaders	Prof. Dr. Dr. Axel Haverich (BREATH) Prof. Dr. Robert Voswinckel (UGMLC)
Participating DZL Partner Sites	BREATH, CPC-M, UGMLC
Number of Participating DZL Faculty	25

Various acute and chronic lung disorders ultimately lead to endstage lung disease (ELD). Once all options for mechanical ventilation have been exhausted, only two treatment options remain for these patients on the brink of death: extracorporeal lung oxygenation (ECMO) and lung transplantation (LTx) Today ECMO therapy remains restricted to short-term application, primarily as bridge to transplantation and as bridge to recovery in acute pulmonary infectious disease (for example, H1N1). In chronic injury, LTx remains the only available therapy with the potential of true long-term survival. LTx, however, is limited to highly selected patients, excludes any pulmonary malignancy, and long-term survival can be severely compromised by chronic rejection. Regenerative therapies that promote endogenous repair, cell transplantation, or tissue engineering are currently not available. The DZL ELD program aims to refine transplantation procedures and to minimize acute and chronic rejection. It also aims to optimize ECMO therapy towards fully implantable devices and set the stage for regeneration of diseased lung tissue.



The Thematic Priority Area "Endstage Lung Diseases" focuses on acute and chronic end-stage pulmonary diseases that do not resond to conventional therapy. The number of patients with terminal pulmonary disease is increasing. This finding is true for acute pulmonary injury from ARDS and infection, chronic disease, pulmonary hypertension, fibrosis, COPD and cystic fibrosis, as well as malignancies.

At the DZL, endstage lung disease is being tackled with a multi-faceted approach by stem-cell researchers, bioengineers, and first-line clinicians and surgeons. LTx, which is often the only option for patients with end-stage lung disease, requires intensive patient care, both before and after transplantion and shapes and drives the urgent need for research in this area.

Goals Followed in 2013 – End-stage Lung Disease

Goal 1 – Lung Transplantation

- Immunology in Lung Transplantation
  - Immunophenotyping of clinical lung transplant recipients before and after LTx
    - Creation of SOPs for FACS of Treg, MDSC and allo-Ab for use at participating locations
    - Monitoring of a regulatory T cell phenotype in PBMC and BAL after LTx
- Immunological tolerance
  - Evaluation of alternative methods for cytoreduction in a porcine lung transplantation model
  - Optimization of alloantigen application in a porcine lung transplantation model
  - Investigation of the mechanism of T cell regulation in a porcine lung transplant model
- Bronchiolitis Obliterans (BOS)
  - New therapeutic strategies for the treatment of neutrophilic inflammation in chronic graft dysfunction after lung transplantation
    - Identification of risk factors and disease-defining variables
    - Development of a flow chart with follow-ups in the LTx cohort
    - Build a database and identify affected patients
    - Follow-up and identification of a cohort (50 min) of LTx candidates with neutrophilic graft dysfunction
    - Identification of new therapeutic strategies in clinical pilot studies
  - Mechanism of BOS
    - Identification of candidate molecules in the murine model of LTx-BOS
    - Investigation of the role of donor and host macrophage activation in BOS-genesis

Goal 2 – ECMO

- ECMO and artificial lung - experimental research
  - Development of biocompatible gas exchange membranes
  - Identification of effective strategies to prevent biofilm formation in the system
  - Development of improved cannulas and cannulation methods for the establishment of suitable methods for connecting extra-and intracorporeal artificial lungs

- Clinical program (lung failure of various origins)
  - Development of a computer-based simulation program for ECMO optimization
  - Development of new cannulation techniques
- Extracorporeal life support in patients with pulmonary hypertension and right heart failure
  - Conduct a clinical study
  - Extraction of tissue samples (pulmonary vessels)

Goal 3 – Regeneration

- iPS ECs for biohybrid ECMO and PH
  - Establishment of endothelial differentiation of iPS cells and characterization of iPS-derived ECs
  - iPS generation from transgenic reporter lines for monitoring of endothelial differentiation and genetic enhancement
  - Optimization of endothelial differentiation and enrichment of the generated iPS ECs
  - Establishment of protocols for the production of iPS with microvascular EC phenotype
- Therapy of lung diseases based on pluripotent stem cells
  - Establishment of respiratory differentiation of human iPS cells and characterization of these cells
  - Human iPS generation of transgenic reporter lines for monitoring the respiratory differentiation and genetic enhancement
  - Execution of screens for the identification of drugs and RNAs which facilitate respiratory differentiation of iPS cells

Goal 4 – Ex Vivo Lung Perfusion

- Use of an innovative ex vivo lung perfusion (OCS) system for the treatment of terminal malignant lung diseases
  - Miniaturization of the system for use in small animals (mouse, rat)
  - Establishment of a tumor model in large animals

Goal 5 - Healthcare Management

- Analysis of the supply situation of patients with terminal lung disease (ELD) and patients after lung transplantation
  - Installation, construction and piloting of the test network; evaluation of the test run; content quality assurance of data
  - Identification of ELD patients; recruitment of appropriate practices for local extension of practice network; complete a basic data set survey and failure analysis
  - Pilot test quality of life and other generic instruments

2013 Research Highlights – End Stage Lung Disease

Research Highlight #1

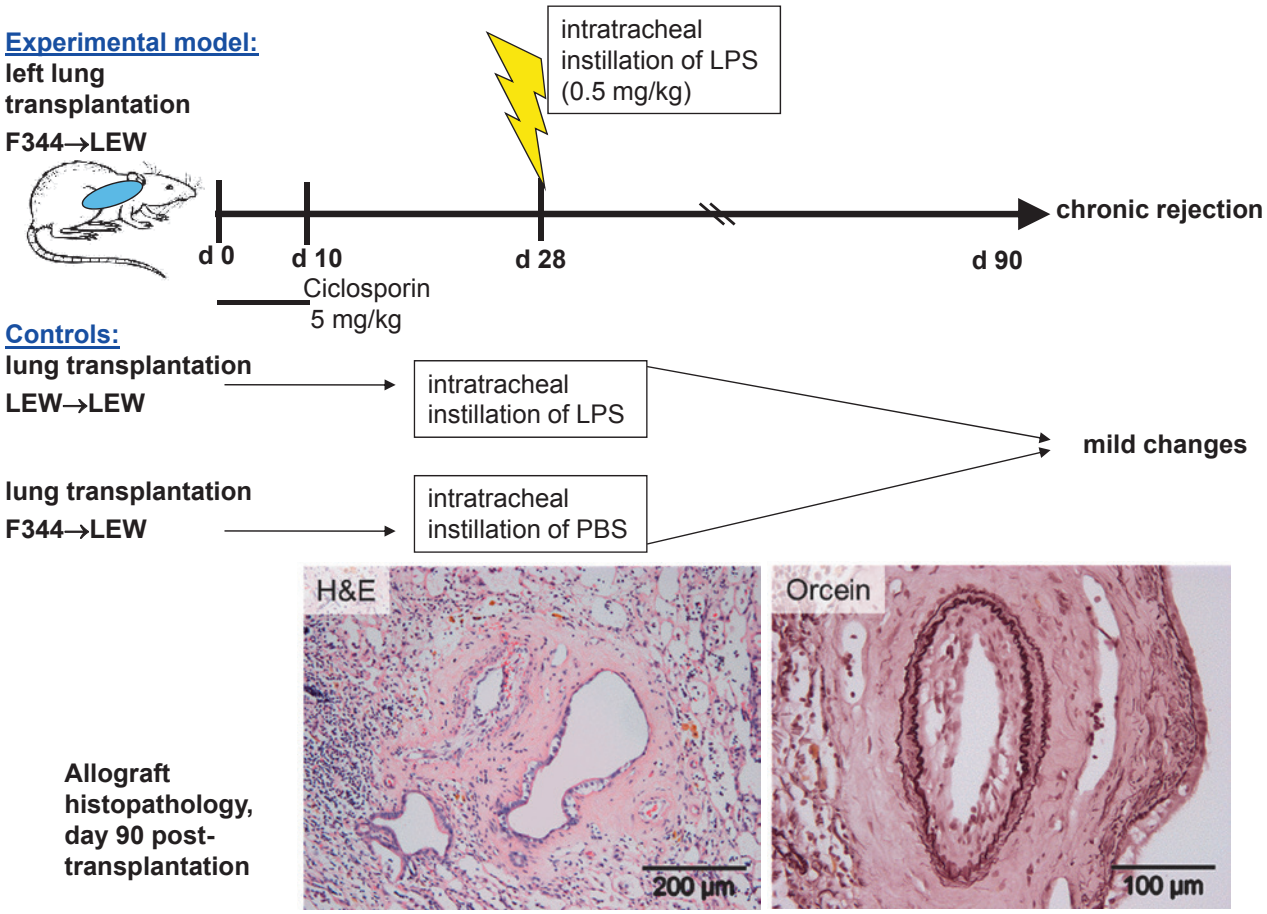


Figure 1. Chronic lung allograft dysfunction/Bronchiolitis obliterans syndrome (BOS) limits the long-term success of pulmonary transplantation. *Main risk factors for BOS are acute rejection episodes and respiratory infection. These risk factors are combined in a novel experimental model where left lungs are transplanted orthotopically in the Fischer-344 (F344) to Lewis rat strain combination. BOS is induced in all allografts but not in isografts in response to lipopolysaccharide (LPS). Both, histopathological hallmarks of human BOS develop (bronchial and vascular remodeling, graft fibrosis) and typical cytokines/chemokines are induced. Allografts treated with vehicle (PBS) rarely develop BOS. This model will help to elucidate the pathogenesis of BOS and to develop innovative therapies. (Atanasova S et al. 2013 J Heart Lung Transplant 32: 1131)*

Research Highlight #2

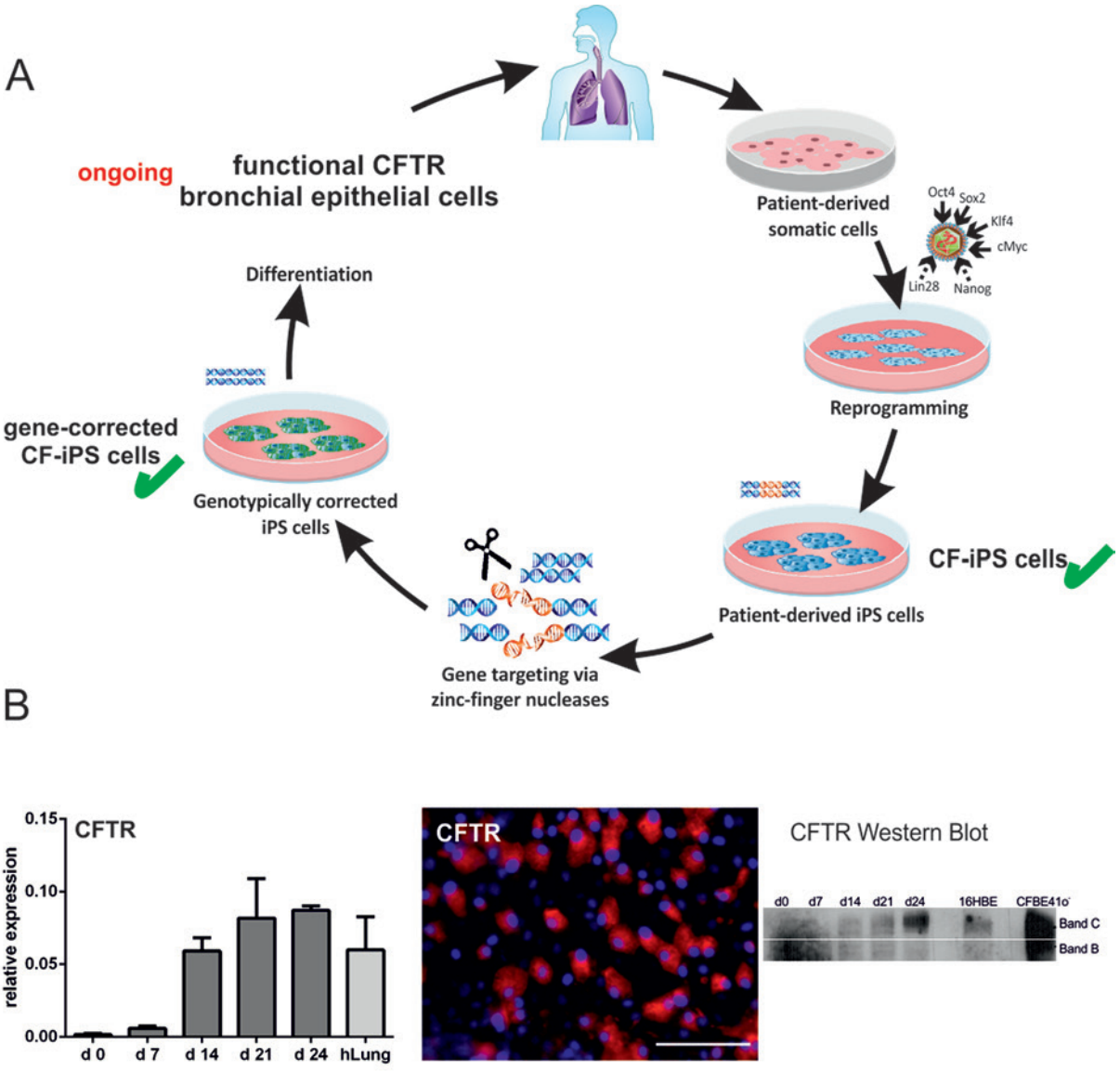


Figure 2. Generation of CFTR expressing airway epithelial cells from patient specific induced pluripotent stem cells. *By reprogramming patient derived somatic cells, human induced pluripotent stem cells (hiPS) with patient and disease specific backgrounds can be generated. Similar to human embryonic stem cells, hiPS show an unlimited potential for proliferation and differentiation in vitro. Novel highly efficient genome engineering approaches allowing for site-specific gene editing in hiPS including Zinc Finger Nucleases (ZFNs) and TALE Nucleases (TALENS) generally provide of the means for producing patient and mutation-specific cell lineages as well as for correcting mutations in the respective cell lines. Targeted differentiation into disease relevant cell types, in the case of cystic fibrosis, into CFTR expressing epithelial cells, could provide cells for in vitro disease modeling, drug screening or future cell therapies (A). Differentiation of human pluripotent cells towards airway epithlelium resulted in CFTR expressing cells, shown by expression analysis (qPCR), immunofluorescence staining as well as western blot with CFTR specific antibodies (B).*

Research Highlight #3

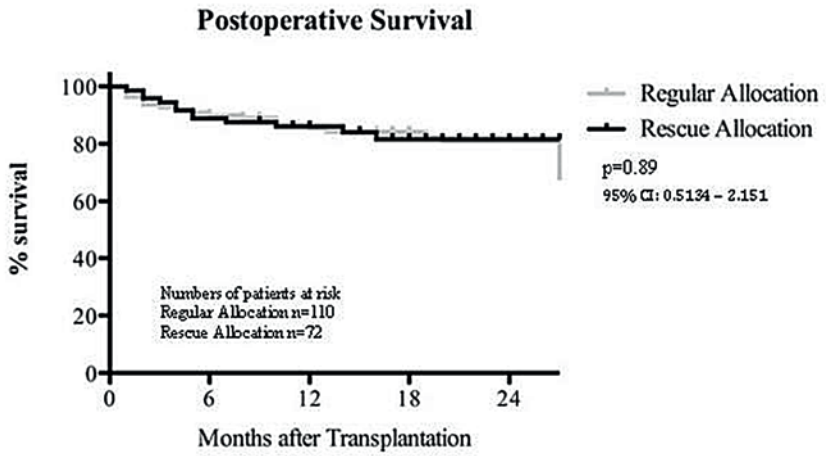


Figure 3. Extended criteria donor lungs and clinical outcome. Lung transplantation is a well-established treatment for patients with end-stage lung disease; however, a lack of suitable donor organs means that timely transplantation is not possible for all patients. Despite the scarcity of donor lungs, many potential donor organs are turned down by transplant centers because no suitable recipient is found according to regular allocation programs. In the publication "Extended criteria donor lungs and clinical outcome results of an alternative allocation algorithm"[1] it was shown that marginal donor organs that had been previously rejected using standard allocation criteria by at least three centers (so called "rescue allocation") could be successfully used for transplant. These marginal donor organs were transplanted into stable "low-risk" patients, whereas the "best" lungs were preferentially transplanted into the most critically ill patients. The authors were able to show comparable short and long-term results in both patient groups, including early and late postoperative morbidity and excellent survival up to two years post-transplantation. Furthermore, by using this approach, the donor pool is noticeably expanded. This practice is especially important since implementation of the Lung Allocation Score (LAS) means that "low-risk" patients with generally low LAS values (such as those with emphysema) no longer have access to the standard donor pool and must therefore be transplanted with organs from the "rescue allocation." This study shows that such transplantation is possible without incurring an increased post-operative risk for these patients. As commented by Prof. John Dark from Newcastle-Upon-Tyne in the UK, this study is "a triumph of intelligent use of these previously "marginal" lungs, giving excellent outcomes from lungs that under some systems might never have been used!"[2]

[1]*Sommer W et al.; Extended criteria donor lungs and clinical outcome: results of an alternative allocation algorithm. J Heart Lung Transplant. 2013 Nov;32(11)*

[2]*Dark J.: Choosing the right lungs for the right patient. J Heart Lung Transplant. 2013 Nov;32(11):1054-5.*

Number of papers published by DZL Faculty in 2013 - Disease Area End-stage Lung Disease: 21

Highlighted Publications

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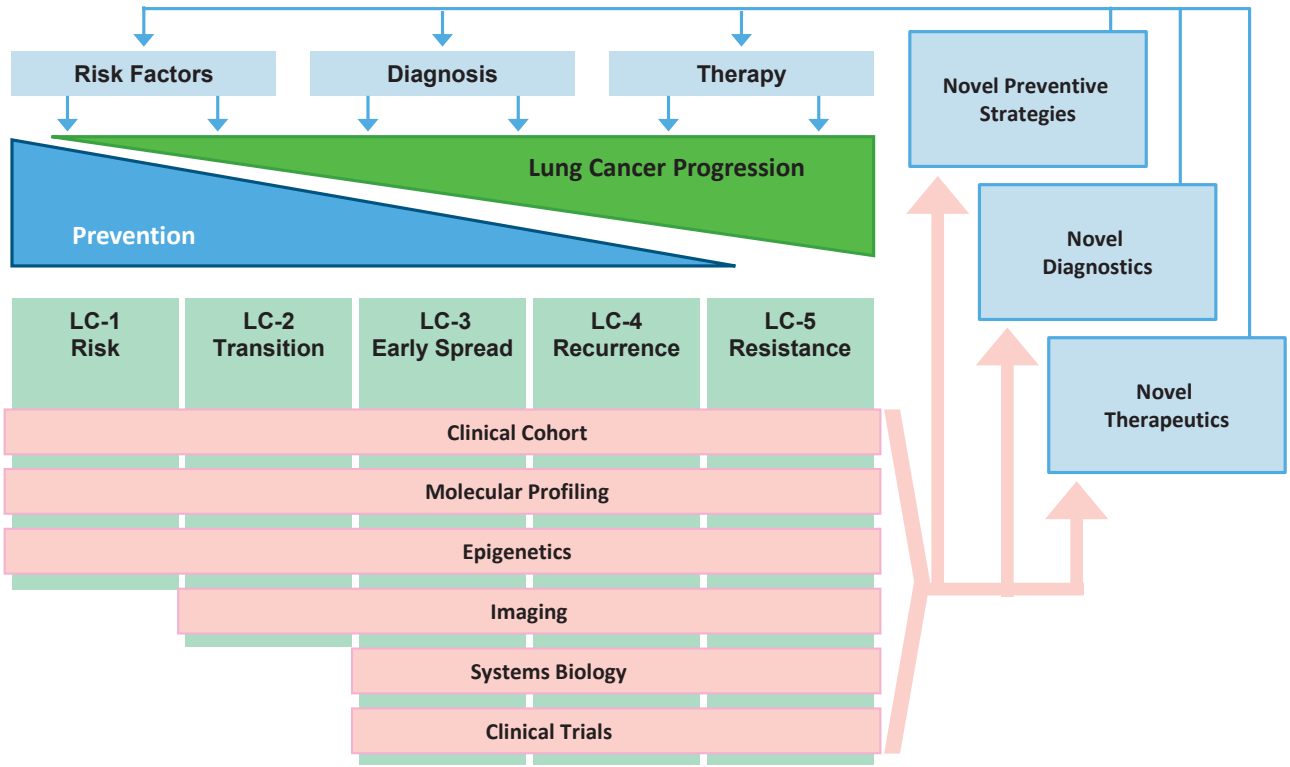
Lung Cancer

Disease Area Leaders	Prof. Ursula Klingmüller (TLRC) Prof. Michael Thomas (TLRC)
Participating DZL Partner Sites	ARCN, BREATH, CPC-M, TLRC
Number of Participating DZL Faculty	31

Lung cancer is a high incidence and high mortality disease. The two main lung cancer types are small-cell-lung carcinoma (SCLC; 20-30% of cases) and non-small cell lung carcinoma (NSCLC; 70-80% of cases). Patients presenting with SCLC have a particularly poor prognosis, and almost 40 % of NSCLC-patients present with metastases at time of diagnosis. Surgery, radiation, chemotherapy, and on a limited scale, targeted treatments –alone or in combination–are used to treat lung cancer. Limited knowledge of which individual molecular markers impact the propagation and spread of the disease impedes the development and use of targeted therapies; hence the treatment success is very variable. Our research focuses on the identification of relevant molecular markers urgently needed to advance matching of targeted treatments to patients, with the ultimate goal of developing personalized therapies to improve patient outcomes. Lung cancer research at the DZL is an interdisciplinary and integrative program exploring clinically well characterized sample sets with epidemiologic, genetic, epigenetic and systems biology approaches.



Goals Followed in 2013 – Lung Cancer

Goal 1 – Epigenetic Markers for Lung Cancer Risk Prediction and Early Detection

- Changes in methylation patterns
  - Optimization of methods for epigenetic analysis
  - Analysis of epigenetic changes and consequences for cell growth
- Epigenetic Lung Cancer Markers
  - Identification of candidate gene list
  - Establishment of a lung cancer risk prediction model
- Clinical validation of epigenetic cancer markers
  - Review of the predictive power of epigenetic markers

Goal 2 – Determinants of Somatic Progression From Airway Epithelium to Lung Cancer

- Carcinogenic stimuli in the lung tissue model
  - Validation of candidate genes using tissue microarray technology (TMA)
  - Identification of hormone receptor binding sites using ChIP-Seq technology
- Comparative analysis of DNA methylation profiles
  - Identification of differential methylation profiles in the transition of COPD to lung cancer
  - Investigation of epigenetic predisposition for lung cancer
  - Biomaterial analysis with probes from fully characterized individuals from a patient cohort
- Clinical validation of transition-defining markers
  - Validation of markers from early screening programs
  - Identification of epigenetic risk factors

Goal 3 – Mechanisms of Early Spread and Predicting Strategies for Intervention

- Dynamics of signal transduction and cell migration in lung cancer cells
  - Quantitative analysis of the TGFbeta, IGF and EGF-induced signal transduction pathways and creation of individual pathway models
  - Analysis of signal transduction at the single cell level and integration into multi-scale model
- Molecular models for improved prognosis
  - Trend analysis determining patterns
  - Validation of prognosis determining molecular patterns
  - Building a patient cohort

Goal 4 – Response and Recurrence in the Combination of Systemic and Radiation Therapy

- Molecular mechanisms of therapy resistance
  - Establishment of integrative dynamic models of repair mechanisms and signal transduction of growth factors
  - Predicting the effects of treatment combinations in vitro

- Characterization of the response to systemic and radiation therapy
  - Analysis of tumor response by morphological and functional imaging
  - Elucidating the mechanisms of therapy resistance
  - Building a patient cohort
- Improved treatment options
  - Development of decision options
  - Identification of targets for maintenance therapy

Goal 5 – Strategies to Mitigate Therapy Resistance

- EGF Receptor signal transduction and resistance mechanisms in preclinical models
  - Identification of resistance mechanisms of EGF receptor signal transduction
  - Development of strategies to overcome resistance based on mathematical models
- Sequential biomaterial collection in metastatic disease
  - Optimization of biomaterial collection, processing, and tissue banking
  - Building a patient cohort
  - Validation of the models predicting development of and overcoming therapy resistance
- Therapy resistance
  - Inspection of molecular targeted therapies in Phase I/II studies with renewed biomaterial acquisition
  - Improving the identification of resistance mechanisms of not yet clinically tested substances

Research Highlight #1

Interleukin-22 is frequently expressed in small- and large-cell lung cancer and promotes growth in chemotherapy-resistant cancer cells. Immunology was long thought to be relatively unimportant in the development of lung cancer. However, a rapidly growing body of evidence has shown that immunological mechanisms significantly influence tumour growth and interaction with normal tissue. This knowledge has opened the door for new therapeutic approaches, some of which are now being tested in clinical trials. In lung cancer, interleukin-22 (IL-22; a signalling molecule of the immune system) expression within primary tissue samples has been found, but the frequency and the functional consequences of IL-22 signalling have not yet been fully addressed. The present study investigated the cellular effects of IL-22 on human lung cancer cell lines. Subsequently, the prognostic impact of IL-22 tissue expression was investigated by immunohistochemistry in 2300 lung cancer patients who had undergone resection of lung cancer. IL-22 serum concentrations were analysed in 103 patients.

The IL-22 receptor 1 was expressed in six of seven lung cancer cell lines. However, IL-22 signalling was functional in only four cell lines, as measured by induced signal transducer activator of transcription 3 phosphorylation (a measure of cell growth) and increased cell proliferation (tissue growth). Furthermore, IL-22 induced the expression of antiapoptotic B-cell lymphoma 2, but did not protect tumour cells from apoptosis (cell death) induced by the chemotherapeutic agent carboplatin. In contrast, cisplatinum-resistant cell lines showed a significant up-regulation of IL-22-receptor 1 (cf. figure) along with a stronger proliferative response to IL-22 stimulation. In the resected tumours IL-22 was preferentially expressed in small- and large-cell lung carcinoma (58% and 46% of cases, respectively). However, in operable stages no correlation between IL-22 expression by immunohistochemistry and prognosis could be observed.

Conclusions: IL-22 is frequently expressed in lung cancer tissue. The enhanced IL-22-receptor 1 expression and the effects in cisplatinum-resistant cell lines suggest that IL-22 stimulates tumour activity and may promote a more aggressive lung cancer phenotype. To further investigate these mechanisms and potential targets for therapeutic intervention, we are currently analysing IL-22 and other cytokines in bronchial lavage specimens and blood samples before and during therapy.

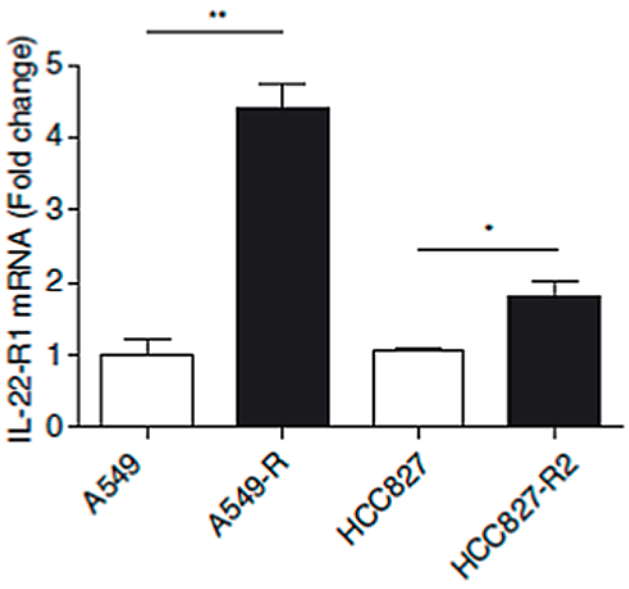


Figure1. IL-22-receptor 1 in cell lines A549 and HCC827. *White columns: cells sensitive to cisplatinum. Black columns: cells resistant to cisplatinum.*

Adapted with permission from Lippincott Williams and Wilkins/Wolters Kluwer Health: J Thoracic Oncology, copyright 2013. Kobold S, Völk S, Clauditz T, Küpper NJ, Minner S, Tufman A, Düwell P, Lindner M, Koch I, Heidegger S, Rothenfußer S, Schnurr M, Huber RM, Wilczak W, Endres S. Interleukin-22 is frequently expressed in small- and large-cell lung cancer and promotes growth in chemotherapy-resistant cancer cells. J Thorac Oncol. 2013 Aug;8(8):1032-42

Research Highlight #2

microRNAs (miRNAs) are small non-coding RNA molecules that represent promising diagnostic and prognostic markers for tumors. Circulating miRNAs were robustly measured in blood samples, which is an important prerequisite for their application as less-invasive biomarkers for diagnosis and prognosis.

Within the DZL, the Thoraxklinik Heidelberg and the German Cancer Research Center (DKFZ) joined forces to analyze the prognostic potential of miRNAs in sera of patients with pulmonary adenocarcinoma. In a genome-wide screen, 10 miRNAs were identified in 40 patient serum samples to be associated with the risk of tumor recurrence after surgery. The potentially prognostic miRNAs were subsequently analyzed in a validation cohort comprising 114 patients. An elevated level of serum miR-142-3p was found to be associated with poor patient prognosis (Figure 2). Importantly, combining data on this putative molecular marker with data on tumor stage further improved the differentiation between low and high risk for recurrence (AUC=0.78, p=0.007; Figure 3). In conclusion, the study showed that serum miRNAs in combination with existing prognostic factors can improve the prediction of recurrence of lung tumor metastases.

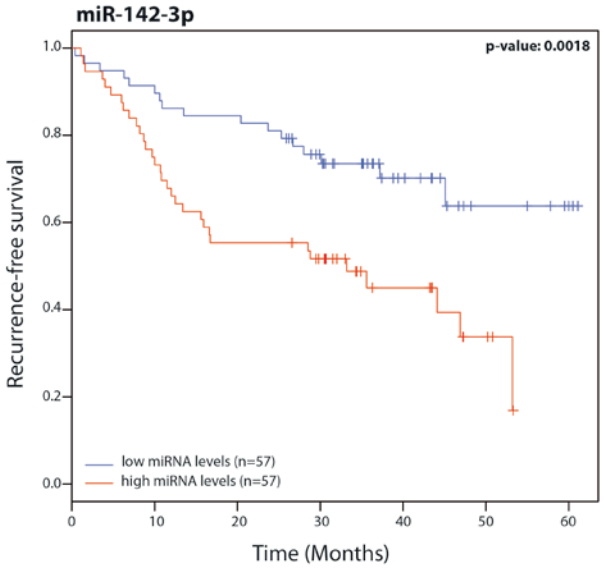


Figure 2: *Poor recurrence-free survival of patients was associated with high miR-142-3p levels (red line)*

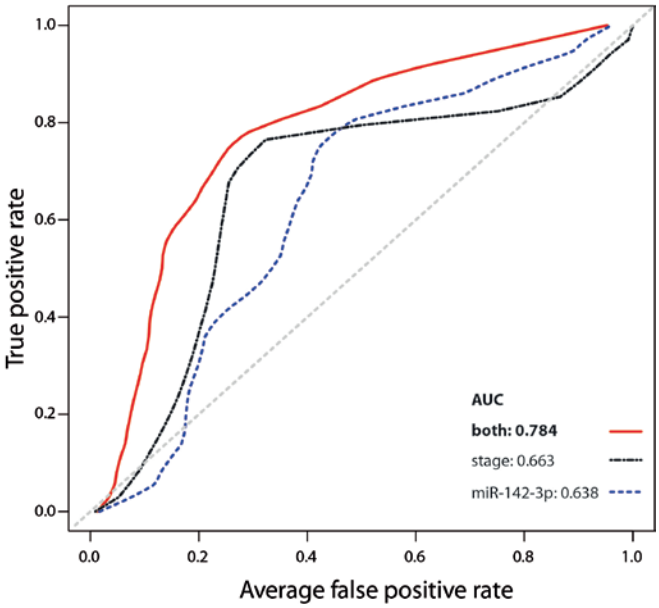


Figure 3: *Improving prediction of recurrence in patients by using miRNA and stage (red line) in comparison to either miR-142-3p levels (blue line) or staging (black line) alone*

Kaduthanam S, Gade S, Meister M, Brase JC, Johannes M, Dienemann H, Warth A, Schnabel PA, Herth FJ, Sültmann H, Muley T, Kuner R. Serum miR-142-3p is associated with early relapse in operable lung adenocarcinoma patients. Lung Cancer 80(2); 223-227, 2013

Research Highlight #3

Randomized phase II trial: Erlotinib vs Carboplatin/ Vinorelbin in elderly patients (> 70 years) with advanced non-small cell lung cancer (TIE study)

Non-small cell lung cancer (NSCLC) is mostly a disease of the elderly. Due to co-existing diseases and reduced relevant organ functions such as renal or hepatic function, the optimal application of systemic therapies remains difficult and the use of oral drugs like the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) erlotinib with a convenient tolerability profile may benefit patients.

In the TIE study, a randomized phase II trial, the efficacy of a monotherapy with erlotinib was investigated against a combination chemotherapy with carboplatin and vinorelbine. 284 mostly Caucasian patients with a median age of 76 years were included. There was a significant superiority in favor of the chemotherapy for progression free survival (PFS), which was the primary endpoint of the trial (median PFS: 2.4 vs 4.6 months, erlotinib vs carboplatin/vinorelbine, p: 0.0005) and a trend in improvement of overall survival (OS) (median OS: 7.3 vs 8.4 months, erlotinib vs carboplatin/vinorelbine, p>0.05). As expected more skin toxicities and more diarrhea were seen in the erlotinib arm and more nausea, neurotoxicities as well as myelotoxicities in the chemotherapy arm. However, the rate of relevant myelotoxities was low (febrile Neutropenia CTC Grade 3/4: 3%). The proportion of patients with EGFR-positive turmors was also low (10%).

In conclusion, this trial confirmed the feasibility and efficacy of platinum based chemotherapy in eligible elderly patients with advanced NSCLC and the inferior efficacy of erlotinib in a population of patients with predominantely EGFR negative tumors.

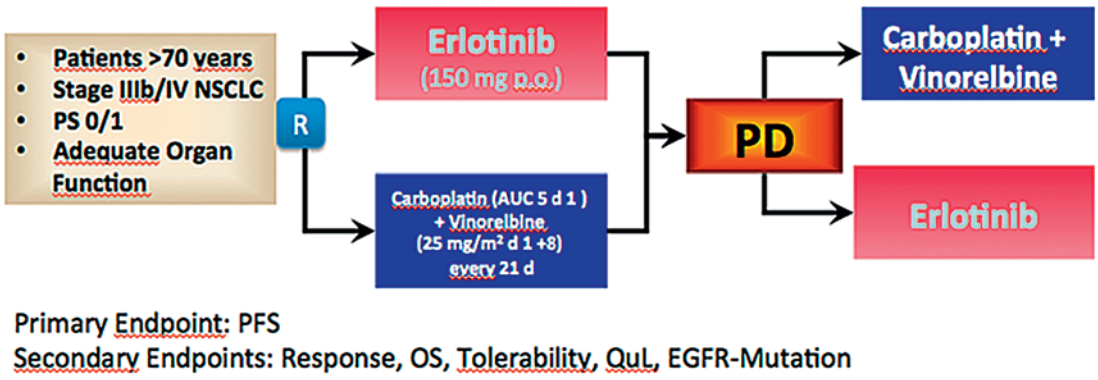


Figure 4: Design and endpoints of the TIE study

Heigener DF, Deppermann KM, von Pawel J, Fischer JR, Kortsik C, Bohnet S, von Eiff M, Koester W, Thomas M, Schnabel P, Reck M. Open, randomized, multi-center phase II study comparing efficacy and tolerability of Erlotinib versus Carboplatin/Vinorelbin in elderly patients (> 70 years) with untreated Non-Small Cell Lung Cancer. Lung Cancer (2014); 84: 62-66.

Number of papers published by DZL Faculty in 2013 - Disease Area Lung Cancer: 77

Highlighted Publications

1. Heigener DF, von Pawel J, Eschbach C, Brune A, Schmittel A, Schmelter T, Reck M, Fischer JR. Prospective, multicenter, randomized, independent-group, open-label phase ii study to investigate the efficacy and safety of three regimens with two doses of sagopilone as second-line therapy in patients with stage iiib or iv non-small-cell lung cancer. Lung Cancer 2013;80:319-325.
2. Kaduthanam S, Gade S, Meister M, Brase JC, Johannes M, Dienemann H, Warth A, Schnabel PA, Herth FJ, Sultmann H, Muley T, Kuner R. Serum mir-142-3p is associated with early relapse in operable lung adenocarcinoma patients. Lung Cancer 2013;80:223-227.
3. Kobold S, Volk S, Clauditz T, Kupper NJ, Minner S, Tufman A, Duwell P, Lindner M, Koch I, Heidegger S, Rothenfuer S, Schnurr M, Huber RM, Wilczak W, Endres S. Interleukin-22 is frequently expressed in small- and large-cell lung cancer and promotes growth in chemotherapy-resistant cancer cells. Journal of Thoracic Oncology : Official Publication of the International Association for the Study of Lung Cancer 2013;8:1032-1042.
4. Kreuter M, Vansteenkiste J, Fischer JR, Eberhardt W, Zabeck H, Kollmeier J, Serke M, Frickhofen N, Reck M, Engel-Riedel W, Neumann S, Thomeer M, Schumann C, De Leyn P, Graeter T, Stamatis G, Zuna I, Griesinger F, Thomas M, investigators T. Randomized phase 2 trial on refinement of early-stage nsclc adjuvant chemotherapy with cisplatin and pemetrexed versus cisplatin and vinorelbine: The treat study. Annals of Oncology : Official Journal of the European Society for Medical Oncology/ESMO 2013;24:986-992.
5. Reck M, Heigener DF, Mok T, Soria JC, Rabe KF. Management of non-small-cell lung cancer: Recent developments. Lancet 2013;382:709-719.
6. Reinmuth N, Stumpf A, Stumpf P, Muley T, Kobinger S, Hoffmann H, Herth FJ, Schnabel PA, Warth A, Bischoff H, Thomas M. Characteristics and outcome of patients with second primary lung cancer. The European Respiratory Journal 2013;42:1668-1676.

Platform Biobanking

Scientific Coordinators	Prof. Dr. Andreas Günther (UGMLC) Dr. Thomas Muley (TLRC)
Participating DZL Partner Sites	ARCN, BREATH, CPC-M, TLRC, UGMLC
Number of Participating DZL Faculty	13

Introduction and aims:

Biomaterials are a key component in translational lung research. Translational research projects can either be undertaken following a "bottom up" (from the test tube to humans) or a "top down" approach (from humans to a cellular mechanism). In both cases, biomaterials are of utmost importance, as they are the basis for the development of new pathomechanistic concepts, innovative therapies and individualized treatment options ("individualized medicine", "targeted therapy"), the identification of prognostically relevant biomarkers, and for the verification of basic science findings.

Goals followed in 2013 – Platform Biobanking

Goal 1 – Build a DZL Biobank Portal

- Regular meetings and teleconferences
- Extension of the DZL Biobank portal

Goal 2 – Harmonization of Procedures and Guidelines

- Identification of existing SOPs
- Harmonization of existing SOPs
- Establishment of DZL Platform Biobanking By-laws

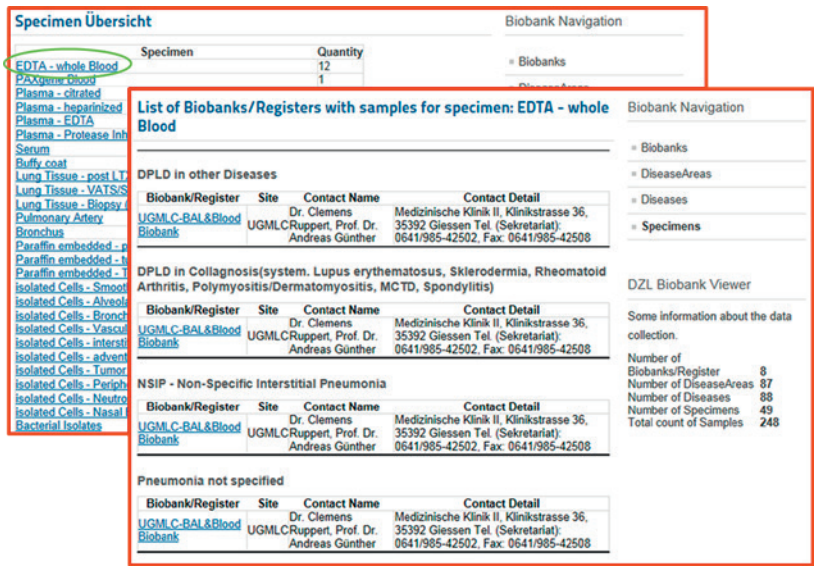
Goal 3 – Harmonization of Phenotyping Tools

- Identification of existing phenotyping tools
- Creation of a centralized, disease independent parameter catalog of bio-material collection, storage and documentation

The centrally-organized DZL-platform “Biobanking” aims to provide easy and direct access to biomaterials from patients with the pulmonary diseases studied within the DZL to DZL members as well as external partners. In order to reach these goals the following achievements have been accomplished:

1. The structure, organization, informed consent procedures, data management and quality control procedures, and biomaterial collections of site-specific, local biobanks have been assessed.

2. A database with retrospectively collected biomaterials has been established, which will be transferred into the “biobanking” website of the DZL-Homepage (www.dzl.de). Every scientist can then inform her/himself on the availability of retrospectively collected biomaterials for a given disease area or disease entity and which formal requirements must be met in order to gain access to these biomaterials. (see figure below)



3. DZL Platform Biobanking “By-Laws” have been developed to administer the handling of biomaterials and data within the DZL as well as with external cooperation partners. The By-Laws are on track for finalization and approval in 2014.

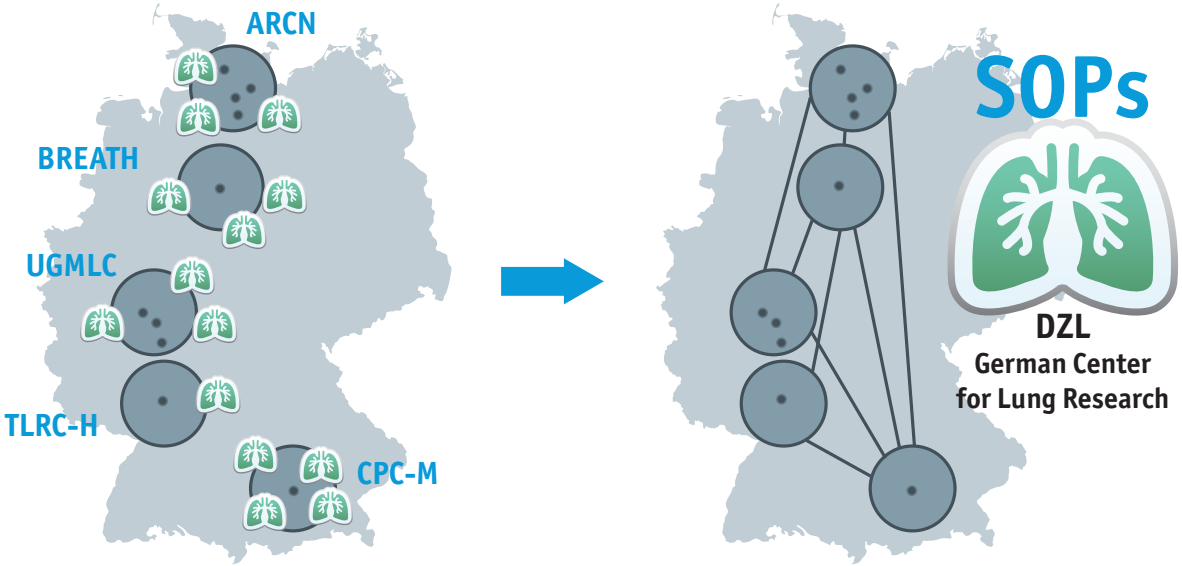
4. In order to be able to collect biomaterials within the DZL under a harmonized informed consent procedure, the platform members have developed a broad consent form that is based on a recent version of a broad consent form developed by the “Arbeitsgruppe Biobanken im Arbeitskreis medizinischer Ethikkommissionen (AK-EK)” which will be the underlying basis for future biobank-based informed consent procedures throughout Germany. This DZL informed consent procedure, alongside with a DZL-wide data protection concept, will be submitted to the first ethics committee mid 2014 and used as the standard informed consent procedure for all further biomaterial collections. The DZL informed consent procedure not only covers the collection and the timely unlimited use of biomaterials but also the acquisition and storage of phenotyping data, includ-

ing radiographic and histological data and the acquisition, storage and use of genetic data including next generation sequencing (deep sequencing). This informed consent procedure will allow easy forwarding and exchange of any data among DZL scientists according to the data protection concept

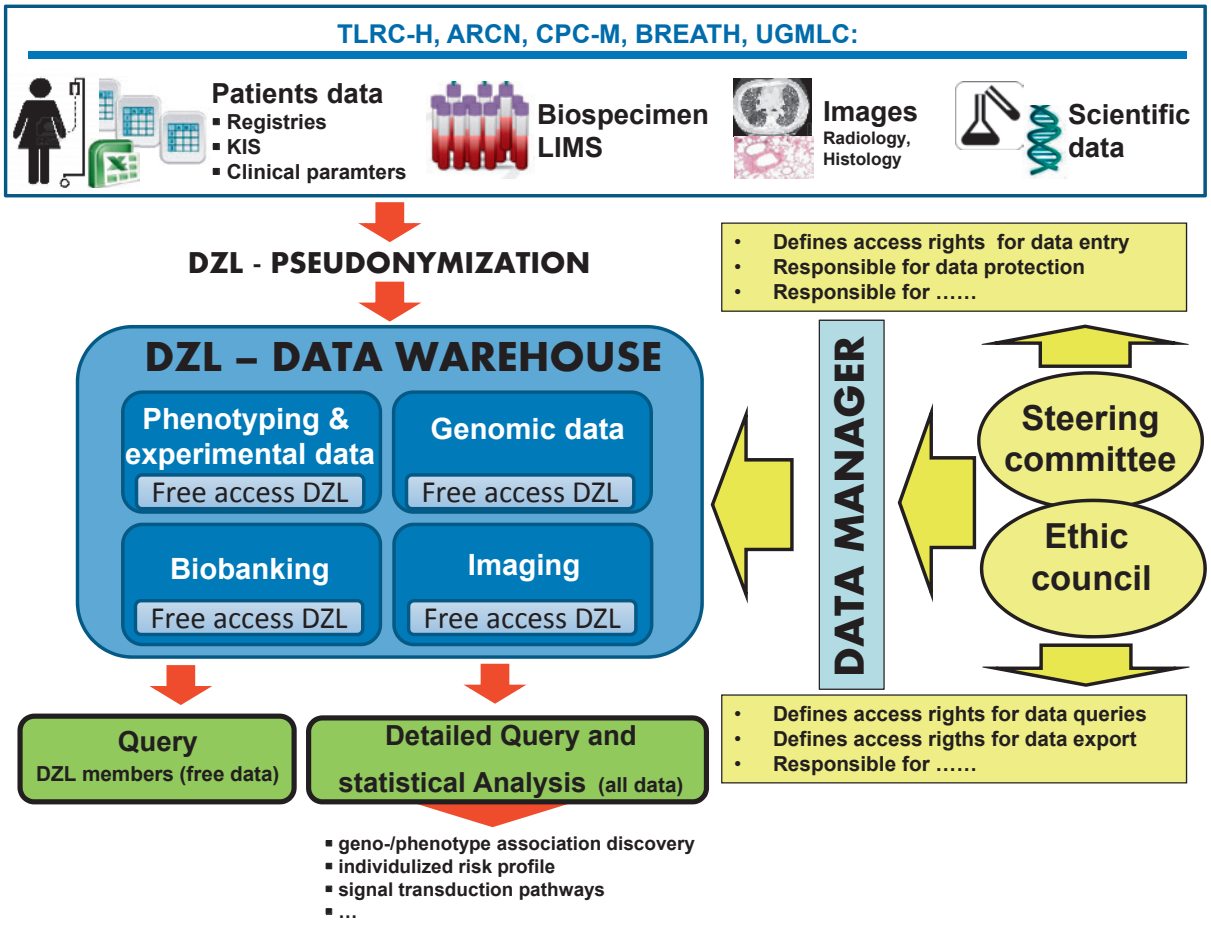
5. The data safety concept, which is based on most recent standards (TMF, data safety protection officers), makes use of a double pseudonymization process, making re-identification of patients impossible. Based on this technique, the generation of site, specimen and date-of-collection-specific LabIDs is possible throughout all DZL sites, allowing the generation of a unique and definite, DZL-wide Lab-ID. The software and hardware structure necessary to provide this service is already implemented. In March 2014 the vote for the data safety concept was committed by the TMF. As soon as the ethic votes have been obtained, the entire DZL structure covering biomaterial acquisition, collection of phenotyping data and data analysis by means of a data warehouse concept will be available.

6. In order to improve the quality of the collected data and biomaterials, the platform biobanking has been systematically reviewing all procedures associated with patient identification, biomaterial withdrawal, primary processing for the various purposes (e.g., RNA extraction, protein-isolation, cell isolation, etc), as well as shipment and secondary steps necessary for definite analysis. A complete list of procedures has been established and serves as underlying basis for the development of DZL-wide SOPs, which will result in a harmonization of the currently SOPs available at different DZL sites. A uniform template has been developed and the first harmonized SOPs have been approved by the DZL Board of Directors.

7. As biomaterials are stored locally, the platform members agreed to use their individual storage and labeling systems. Most DZL members use 2D-barcode tubes from different suppliers and different laboratory information and management systems (LIMS) for sample registration and tracking. As outlined above, samples collected under a harmonized DZL informed consent will be additionally labeled with a central DZL-unique sample ID.



8. Phenotyping data will be either collected in the frame of DZL-associated registries (e.g. COSYCONET, eurIPF-net) or phenotyping tools or by using special tools to be developed for the DZL. The double pseudonymization process will allow samples to be linked with phenotyping data (biometric data, family history, occupational history and disease-specific clinical parameters). To facilitate this process and to be able to integrate data from several DZL resources and to also include research data as reasonable (e.g. all ~ omics datasets), an overarching data warehouse system was developed, which is based on i2b2 (Informatics for Integrating Biology & the Bedside) as a data warehouse system. The proposed IT-structure and IT management concept will be established according to the figure below.



Platform Imaging

Scientific Coordinators	Prof. Dr. Hans-Ulrich Kauczor, TLRC Prof. Dr. Matthias Ochs, BREATH Prof. Dr. Heinz Fehrenbach, ARCN
Participating DZL-sites	ARCN, BREATH, CPC-M, TLRC, UGMLC
Number of participating DZL faculty	22

Overview

A wide range of imaging approaches is used in the life sciences to understand living systems and to support the drug discovery processes. The Platform Imaging has been established as a network of complementing expertise and infrastructure within the DZL to ensure scientific exchange and access to cutting-edge imaging technologies in research. Comprising radiology and microscopy, the platform imaging aims to identify and benefit from the interfaces between them. The core function of the platform is to offer, disseminate, and share imaging technology.

The platform is managed by the central coordination office at the Translational Lung Research Centre in Heidelberg (TLRC). Interactions between the Platform Imaging and the DZL disease areas are supported by activities to enhance visibility, transparency and communication.

Visibility

The Platform Imaging uses the DZL-website to inform DZL members and external researchers about its mission, core tasks and services offered.

Transparency

A number of documents including the lists for radiologic and microscopic equipment, the bylaws of the Image Bank and the minutes of formal meetings and telephone conferences are available to registered users via the DZL-Intranet.

Communication

DZL-Principal Investigators and their project collaborators have been invited to join the e-mail distribution list of the platform to be always informed about actual developments and events. Almost 100 DZL-members from all the sites and Disease Areas subscribed in 2013. A contact list for radiology and microscopy experts at each DZL-site has been collated and can be downloaded from the DZL website.

Goals followed in 2013 - Platform Imaging

Goal 1 - Framework

- Maintenance of the E-Mail distribution list
- Regular update of the equipment list
- Establishment of regular telephone conferences
- Development of a concept for annual meetings and conduct of the first two-day meeting for all members of the Platform Imaging in Giessen

Goal 2 – Basic Documents

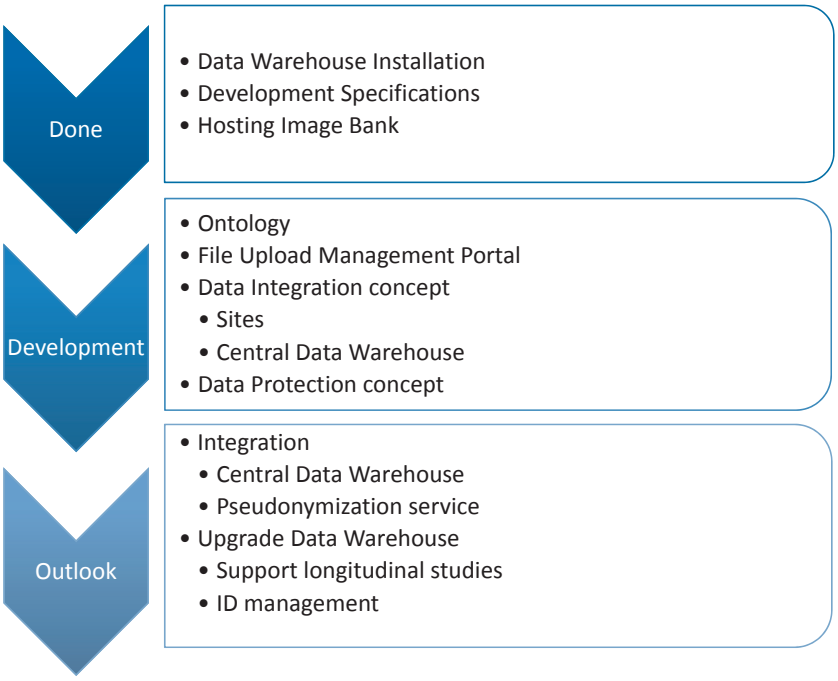
- Bylaws of the Image Bank
- Price list with recommendations for the use of CT and MRI in prospective, multicenter clinical trials.

Goal 3 - Exchange of Methodologies

- Development of a procedure for the exchange of methodologies based on the documents, the regular telephone conferences, meetings, workshops and webinars

Goal 4 – Image Bank

- Installation of i2b2 is completed



Core project of the Platform Imaging: Image Bank

Figure 1 Overview Image Bank 2013

In 2013, various concepts were developed for the data management of the Image Bank.

For the purpose of future integration of the Image Bank into the central DZL Data Warehouse (see Platform Biobank above), the web-based i2b2 tool (Informatics for Integrating Biology & the Bedside) was installed as data warehouse solution for the Image Bank. The necessary i2b2 ontology for structuring the data was derived from the minimum dataset previously agreed on. The data management solutions developed also consider the data collected within longitudinal clinical studies.

The data exchange between the DZL sites and the Image Bank is carried out by an upload file portal. The connected Image File Management Portal verifies the correct use of pseudonymization of the data and indexes the data before proceeding with the upload. In addition, functions are implemented in the Image File Management Portal to initiate automated quantitative analysis (post-processing).

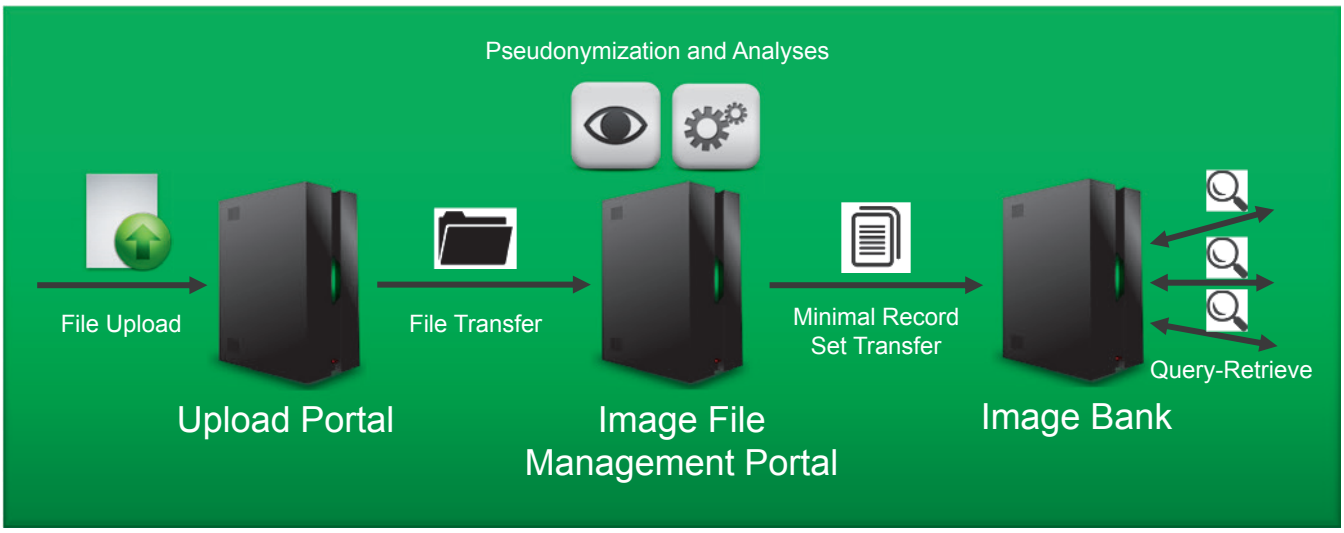


Figure 2 Process file upload to indexing in Image Bank

Research Highlight #1: Microscopy

Multiplex Profiling of Cellular Invasion in 3D Cell Culture Models.

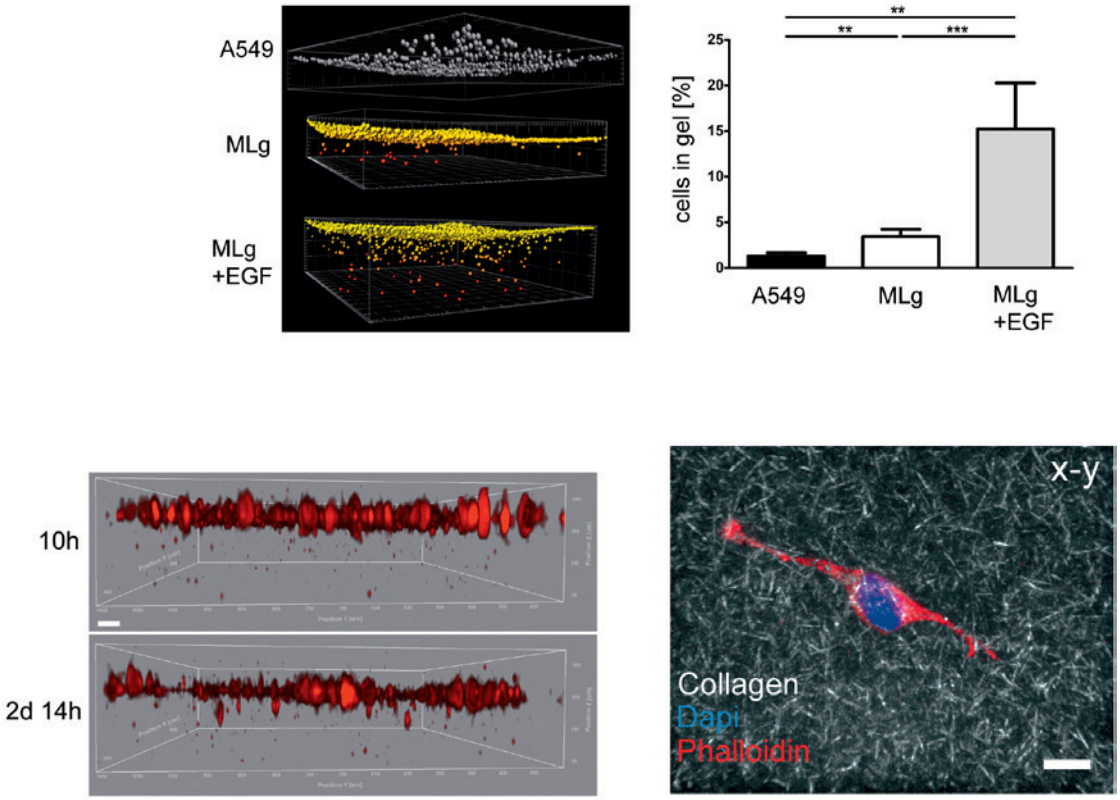


Figure 3. Aberrant fibroblast tissue invasion plays a pivotal role in neoplastic and non-neoplastic diseases including cancer and interstitial lung disease. *To investigate cellular invasion in detail, a novel high-content 3D collagen-based cell culture assay was developed. By seeding cells on top of a collagen-gel in 96-well plates and allowing them to invade the gel for 72 hours, one can study the invasive capacity of cells by a software-based evaluation of the number of cells that invaded the collagen matrix. Treatment of the fibroblastic cells with epidermal growth factor (EGF) further augmented the invasiveness of these cells. These results were corroborated by confocal 4D live-cell imaging of invading fibroblasts. A separation of invading from non-invading cells allowed the molecular analysis of the invasive phenotypes.*

References: Burgstaller G, Oehrle B, Koch I, Lindner M, Eickelberg O. Multiplex profiling of cellular invasion in 3D cell culture models, PLoS One. 2013 May 9;8(5):e63121.

Research Highlight # 2: Radiology

Pulmonary Emphysema Diagnosis with X-ray Dark-Field Imaging

Pulmonary emphysema is one of the leading causes of morbidity and mortality worldwide. The changes in lung tissue morphology are difficult to detect with conventional x-ray absorption imaging techniques, particularly at early stages.

In the presented proof-of-principle study, transmission, phase-contrast, and dark-field scatter-contrast images of murine lungs were obtained with a novel compact cone-beam grating-based prototype scanner. Microscopic pathology-induced structure changes of the lung tissue could be detected via analysis of the joint distribution of transmission and dark-field signal. Thus, the combination of dark-field scatter radiography with conventional radiography can offer new non-invasive diagnostic access for the diagnosis of emphysema.

The referenced investigations were conducted in a multidisciplinary approach funded by the DZL, the Munich-Centre for Advanced Photonics (DFG) and the European Research Council (ERC).

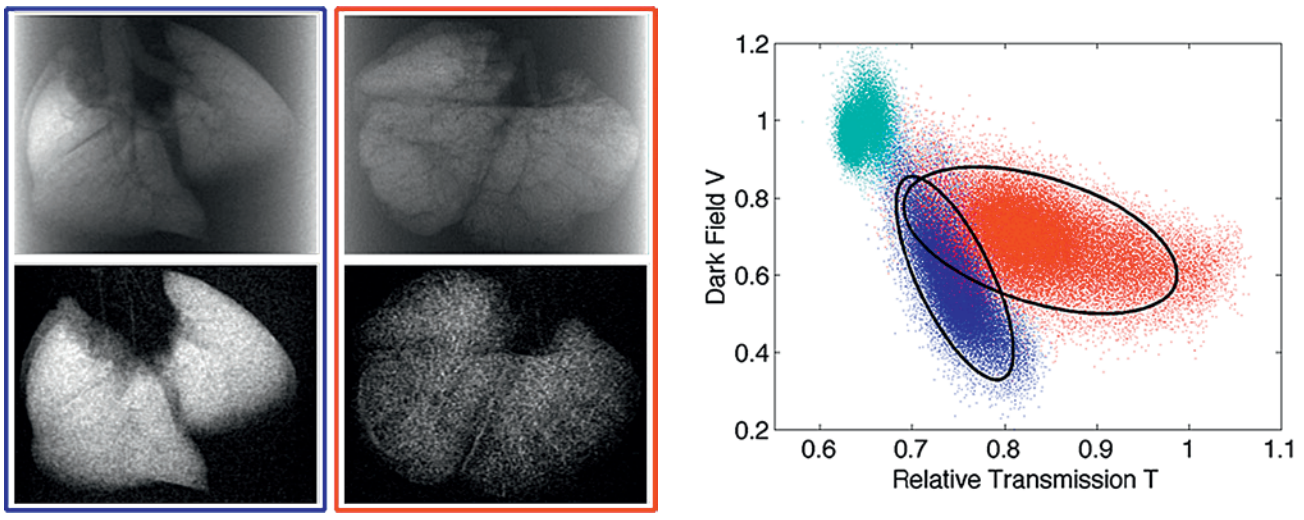


Figure 4. A healthy (left, blue) and an emphysematous (right, red) lung can be seen on the figure in the conventional transmission (top) and dark-field (bottom) imaging modality. *While it is very challenging to detect the pulmonary disorder relying on the conventional transmission signal alone, a much clearer distinction can be achieved with dark field. However, the best discrimination is obtained if the two signals are combined. The scatter plot to the right shows the signals for healthy (dark blue) and emphysema (red) tissue, obtained for three healthy and three emphysematous lungs.*

References:

1. Yaroshenko, A. et al. Pulmonary Emphysema Diagnosis with a Preclinical Small-Animal X-ray Dark-Field Scatter-Contrast Scanner. Radiology (2013).

2. Schleede, S. et al. Emphysema diagnosis using X-ray dark-field imaging at a laser-driven compact synchrotron light source. Proceedings of the National Academy of Sciences 109, 17880–17885 (2012).

Number of papers published by DZL Faculty in 2013 – Platform Imaging: 57

Highlighted Publications – Microscopy:

1. Hussong J, Lindken R, Faulhammer P, Noreikat K, Sharp KV, Kummer W, Westerweel J. Cilia-driven particle and fluid transport over mucus-free mice tracheae. Journal of Biomechanics 2013;46:593-598.
2. Muhlfeld C, Ochs M. Quantitative microscopy of the lung: A problem-based approach. Part 2: Stereological parameters and study designs in various diseases of the respiratory tract. American Journal of Physiology Lung Cellular and Molecular Physiology 2013;305:L205-221.
3. Ochs M, Muhlfeld C. Quantitative microscopy of the lung: A problem-based approach. Part 1: Basic principles of lung stereology. American Journal of Physiology Lung Cellular and Molecular Physiology 2013;305:L15-22.

Highlighted Publications – Radiology:

1. Lederlin M, Puderbach M, Muley T, Schnabel PA, Stenzinger A, Kauczor HU, Heussel CP, Herth FJ, Hoffmann H, Dienemann H, Weichert W, Warth A. Correlation of radio- and histomorphological pattern of pulmonary adenocarcinoma. The European Respiratory Journal 2013;41:943-951.
2. Vogel-Claussen J, Renne J, Hinrichs J, Schonfeld C, Gutberlet M, Schaumann F, Winkler C, Faulenbach C, Krug N, Wacker FK, Hohlfeld JM. Quantification of pulmonary inflammation after segmental allergen challenge using turbo-inversion recovery-magnitude magnetic resonance imaging. American Journal of Respiratory and Critical Care Medicine 2014;189:650-657.
3. Wielputz MO, Weinheimer O, Eichinger M, Wiebel M, Biederer J, Kauczor HU, Heussel CP, Mall MA, Puderbach M. Pulmonary emphysema in cystic fibrosis detected by densitometry on chest multidetector computed tomography. PloS One 2013;8:e73142.

DZL Clinical Trial Board

In 2013 as in 2012, the DZL funded investigator initiated innovative clinical trials through a competitive application process. The trials funded upon recommendation of the DZL Clinical Trial board in 2013 are the following:

Coordinating PIs	Disease Area	Title
Behr/Günther	DPLD	Exploratory efficacy and safety study of oral pirfenidone for progressive, non-IPF lung fibrosis (RELIEF in lung fibrosis)
Seeger/Voswinckel	COPD	Palifermin inhalation as add-on therapy for advanced COPD and emphysema (inhalation toxicology study funded only)
Kauke/Winter/ Neurohr/Schramm	End-stage Lung Disease	Impact of de-novo donor-specific antibodies on short- and long-term survival following single and double lung transplantation
Herold/Lohmeyer	Pneumonia and Acute Lung Injury	Promotion of host defense and alveolar barrier regeneration by inhaled GM-CSF in patients with pneumonia-associated ARDS
Lindner	Lung Cancer/ End-stage Lung Disease	Phase II study of pleurectomy/decortication and hyperthermic intrathoracic chemotherapy (HITHOC) or hyperthermic saline lavage for mesothelioma

The clinical trials funded in 2012 were in the disease areas Cystic Fibrosis, Lung Cancer, and COPD and are ongoing.

Members of the DZL Clinical Trial Board are Prof. Dr. Norbert Krug (BREATH), Prof. Dr. Hossein Ardeschir Ghofrani (UGMLC), Prof. Dr. Jurgen Behr (CPC-M), Prof. Dr. Klaus F. Rabe (ARCN) and Prof. Dr. Michal Thomas (TLRC)

DZL Technology Transfer Consortium

An aim of the DZL is to successfully transfer research results into clinical developments for the benefit of patients. This transfer ultimately requires transforming those developments into commercial applications. Intellectual property (IP) is a key component of the commercialization process and the DZL is committed to strategic management of its IP.

Although seemingly straightforward, the management of IP is a challenge due to the structure of DZL as a membership association (eingetragener Verein, e.V.) with a large number of independent partners with different organizational and legal structures. While the interests of DZL have to be secured, it is at the same time important to respect and consider the independence of its member institutions and their manifold organizational and legal structures.

In order to ensure a systematic and effective exploitation of research results, the DZL founded the DZL Technology Transfer Consortium in 2013. The DZL Technology Transfer consortium consists of the technology transfer organizations of all partner institutions of DZL and is chaired by Dr. Christian Stein, Managing Director of Ascenion, and Dr. Peter Stumpf, Managing Director of TransMIT. DZL Internal contacts for the Technology are Prof. Dr. Werner Seeger (UGMLC) and Dr. Annegret Zurawski (BREATH). The institutions participating in the DZL Technology Transfer Consortium are:

- Ascenion
- German Cancer Research Center, Stabsstelle Technologietransfer
- EMBLEM Technology Transfer
- Fraunhofer-Gesellschaft, Abteilung Patente und Lizenzen
- Max-Planck-Innovation
- technology transfer heidelberg
- TransMIT

The aim of the consortium is to be “one face to the customer” for both science and industry and to ensure efficient and effective capitalization of DZL IP. In addition to providing training for scientists about how to identify and protect possible inventions, the DZL Tech Transfer Consortium offers the following services:

- Collection of information regarding invention disclosures, patent applications, type and number of license agreements, and amount of technology transfer related revenue at DZL partner institutions
- Abstract screening services for DZL meetings
- Abstract screening “hotline” for DZL scientists on an as-needed basis
- Exploitation contract review
- Providing counsel regarding preparation for scientific advice meetings with BfArM with the aim of minimizing potential regulatory failures

ascenion

EMBLEM TECHNOLOGY TRANSFER

dkfz. DEUTSCHES KREBSFORSCHUNGSZENTRUM IN DER HELMHOLTZ-GEMEINSCHAFT

Fraunhofer

Max-Planck-Innovation

technology transfer heidelberg

TransMIT Gesellschaft für Technologietransfer mbH

Cooperation and Collaboration

2nd DZL Annual Meeting

The 2nd DZL Annual Meeting was held in Bad Nauheim on January 29 & 30. During a packed two days of meetings, more than 300 DZL scientists came together to discuss the latest developments in the Disease Areas and Platforms studied by the DZL. Six members of the DZL International Advisory Board were present to give feedback and advice to the DZL administration, as well as to participate in the judging of the first annual DZL Poster Contest. During two poster sessions, more than 100 posters were prepared and presented by DZL researchers. The 2013 DZL poster award winners were:

- Elie El Agha - UGMLC
- Christina Mauritz - BREATH
- Rafiq Amir - UGMLC
- Florian Veit - UGMLC
- Michael Wanzel - UGMLC

CAPNETZ

The DZL welcomed the CAPNETZ Stiftung into the DZL as an associated partner. CAPNETZ (Network of Excellence, Community Acquired Pneumonia) is a German-based research network which supports scientific work on community-acquired pneumonia (CAP) and other acute infections of the lower respiratory tract. CAP is a significant problem. In Germany alone it is estimated that 800,000 people per year contract CAP, with as many of a third of them requiring hospitalization. Pneumonia is the sixth leading cause of death in Germany. The aims of CAPNETZ are the optimization of the treatment of patients with CAP and the development of therapeutic recommendations and guidelines. Data are collected on pathogens and pathogen resistance as well as information on diagnostics and therapy of CAP patients. The CAPNETZ database includes information and biosamples from more than 10,000 patients and is the most comprehensive CAP database in the world.

National Collaborations

The DZL is involved in several national research associations. In 2013 the DZL became a full member of the Technology and Methods Platform for Network Research in Medicine (TMF e.V.). DZL scientists actively participated in workshops and symposia offered by the TMV e.V. and took full advantage of this resource. In addition, the DZL cooperates extensively with the German Society for Pneumology (DGP). Three DZL-board members are on the International Advisory Board of the DGP Journal "Pneumologie," and the latest DZL news is frequently published in the journal. In March 2013 the DZL had an information stand at the 54th DGP Congress in Hannover and was involved in several presentations at the meeting. In addition, the DGP established a stipendium for young researchers at centers outside the DZL to bring them to DZL labs for 3 month stays. The first recipient of the DZL DGP Stipendium was Dr. Christoph Tabeling of Charité - Universitätsmedizin Berlin. He worked in the laboratory of Prof. Dr. Norbert Weissmann of the DZL UGMLC.

International Collaboration – DZL German French Lung School

The German French Lung School was officially launched in September 2013. The establishment and promotion of a joint German-French Lung School is an initiative promoted by both the French and German Ministries for Research. A partnership between the French Institute of Health and Medical Research (Institute National de la Santé et de la Recherche Médicale, INSERM) and the DZL, the aim of the German-French Lung School is to facilitate the exchange and common research efforts of graduate students and postdoctoral scholars in France and Germany. Through the creation of this program, students and post-docs engaged in lung research in France and Germany have the opportunity to learn new techniques, be exposed to different ways of scientific thinking and approach, and importantly, to build a network of international contacts that will provide the foundation for success in their careers moving forward.

Five postdoctoral fellowship positions in Germany, one at each DZL Center are available, with 50% of the funding coming from the BMBF and 50% coming from the lab in which the postdoctoral fellow is working. A matching number of postdoctoral positions has been promised in France by INSERM. In addition, participants in the German-French Lung School are eligible to participate in a joint CPC/INSERM retreat.

German French Lung Retreat 2013

From September 16 to 19, 2013, approximately 190 doctoral students, postdoctoral fellows and senior scientists from INSERM (the French Institut national de la santé et de la recherche médicale) and the German Center for Lung Research (DZL), including those participating in the newly established DZL German-French Lung School, met in Tours, France, for the 2nd German-French Lung Retreat co-organized by INSERM and the CPC Research School.

The program consisted of oral presentations and poster sessions on topics such as asthma & allergy, COPD & emphysema, lung inflammation or fibrotic lung disease, and gave young scientists the opportunity to present their research projects and discuss these highly relevant scientific issues with all the attendees. The social program in the nice student city of Tours facilitated networking amongst the attendees.

Tandem projects – DZL German French Lung School

Projects at the CPC-M and UGMLC DZL Centers initiated in 2013. The other three projects are scheduled to start in 2014.

ARCN
- Project Theme: Severe Adult Asthma
- PI Germany: Klaus F. Rabe, LungenClinic Grosshansdorf, Großhansdorf, Germany
- PI France: Pascal Chanez, Département des Maladies Respiratoires, AP-HM, Laboratoire d'immunologie INSERM CNRS U 1067, UMR 7733, Aix Marseille Université, Marseille, France

BREATH
- Project Theme: Non-proteolytic Functions of Alpha-1-Antitrypsin
- PI Germany: Tobias Welte/Sabine Janciauskiene, Medizinische Hochschule Hannover, Hannover, Germany
- PI France: Gabriel Thabut: Service de Pneumologie B et Transplantation Pulmonaire, Hôpital Bichat, Paris, France.

CPC-M
- Project Theme: Functional and morphological abnormalities of bronchial and alveolar epithelial cells in chronic obstructive pulmonary disease (COPD)
- PI Germany: Oliver Eickelberg, Comprehensive Pneumology Center, Helmholtz Center, Munich, Germany
- PI France: Marina Pretolani/Michel Aubier, Inserm U700 – Physiopathology and Epidemiology of Respiratory Insufficiency, Paris, France

TLRC
- Project Theme: Imaging and quantitative analysis of the airways and airway remodeling in chronic obstructive lung diseases
- PI Germany: Hans-Ulrich Kauczor, University Clinic Heidelberg, Heidelberg, Germany
- PI France: François Laurent, Unité d'Imagerie Thoracique et Cardiovasculair, Hôpital Cardiologique du Haut-Lévêque, Pessac, France

UGMLC
- Project Theme: Epigenetic mechanisms in inflammation and vascular remodeling of pulmonary arterial hypertension
- PI Germany: Soni Savai Pullamsetti/Werner Seeger, Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany
- PI France: Frédéric Perros/Marc Humbert, Inserm Univ. Paris-Sud UMRS 999, Centre Chirurgical Marie Lannelongue, Le Plessis Robinson, France

Youth Development and Equal Opportunities

The DZL is committed to training and promoting the careers of young researchers. In addition to DZL Junior Research Group Leader, W2 Professorships and the German-French Lung School, there are several programs designed to support promising young lung researchers. Graduate and medical training programs are available at each DZL Center and young DZL scientists are actively involved in national and international conferences both within and external to the DZL.

Training Programs

ARCN

- Graduate Centers at Universities of Kiel and Lübeck
- Graduate programs from DFG Excellence Initiative that include basic lung research topics such as
  - Modulation of Immunity
  - Genes, Environment, Inflammation
- Borstel Biomedical Research School (BBRS)
  - Fully devoted to lung research
  - Includes scientific topics, soft skills, and mentoring

BREATH

- Hannover Biomedical Research School (HBRS) Programs including
  - Molecular Science
  - Regenerative Medicine
- HBRS Structured Medical Doctors' Program (StrucMed Program)
  - Opportunity for 9 months laboratory research
  - Scientific lectures, soft-skill courses, and mentoring
- All students have access to BREATH's quarterly DZL colloquia

CPC-M

- CPC Research School „Lung Biology and Disease"
  - International, interdisciplinary MD/PhD program
  - Investigates lung biology and disease at the interface of basic science and clinical medicine
  - Three year program, includes scientific curricula, mentorship, and cutting edge research projects
- Munich Medical Research School (MMRS)
  - Central institution of the LMU's Medical Faculty, umbrella structure for all doctoral studies
  - PhD program for medical doctors
- Helmholtz Graduate School Environmental Health (HELENA)
  - Scientific Training in Lung Biology, Lung Diseases and related fields
  - Career promotion and training in management, leadership and communication

TLRC

- Hartmut Hoffmann-Berling International Graduate School of Molecular and Cellular Biology
  - Includes scientific core courses, state-of-the-art life science technologies, soft skills, and mentoring
- Research projects in TLRC labs
- Monthly TLRC research seminars with DZL internal and external speakers

UGMLC

- UGMLC School umbrella program for training of pulmonary scientists at all three UGMLC institutions
- Justus Liebig University Giessen: Molecular Biology and Medicine of the Lung Program (MBML Program)
- Max Planck Bad Nauheim: International Max Planck Research School for Heart and Lung Research (IMPRS-HLR)
- UGMLC School also includes soft skills and mentoring opportunities

Equal Opportunities

Measures to ensure equal opportunities are carried out in close cooperation with the appropriate bodies of DZL partner sites. In the context of gender equality programs of the participating university partners and others, priority is placed on the active recruitment of female scientists to the DZL at every level – from the trainee to the advisory board member.

Lung Information Service

Lung Information Service

The Lung Information Service (Lungeninformationsdienst, LID) is an essential part of the DZL. Headquartered at the Helmholtz Zentrum München, the LID makes new research findings and patient information available to the general public. Launched in 2011, the LID has continued its upward trajectory and its mission of disseminating information about lung diseases to the public. In 2013 the LID published more than 120 news articles on its online portal and has more than 1800 subscribers to its monthly newsletter and/or individual RSS feeds. An important source of LID information is articles published on patient-relevant topics in top journals, including an increasing proportion with DZL-authorship. In addition, the LID regularly publishes expert interviews on current issues in lung research, including in 2013 interviews with DZL scientists Prof. Tobias Welte (BREATH), Prof. Klaus F. Rabe (ARCN), and Prof. Jürgen Behr (CPC-M). The LID also publishes on special topics, such as pneumonia, end-stage lung diseases, as well as rare lung conditions such as Kartagener syndrome or lymphangioleiomyomatosis. In addition to purely scientific content, the LID publishes information about patient-relevant events, literature recommendations to patients, and announcement of lung-relevant television and radio broadcasts.

The number of visitors to the online portal www.lungeninformationsdienst.de increased in 2013 from 21,700 monthly unique visitors in January to 41,000 in November and the Google ranking of the LID has also significantly improved.

With the aim of informing patients who do not have access to the online portal, the LID has launched a new publication series. Called “The Essentials”, these publications are two-sided fact sheets in durable, plastic-coated, “doctor’s coat format” which summarize the current state of knowledge on individual diseases, diagnostic methods, therapeutic approaches and other important issues. The first five topics addressed were pollen allergies, COPD, asthma, pulmonary fibrosis, and medicines for lung disease.

Patient information forums are also an important part of the LID. In 2013 the LID hosted two patient information forums in Munich. The first event was held in July and covered the topic “inflammatory processes in chronic lung diseases.” Participating DZL faculty were Dr. Claus Neurohr (CPC-M), Prof. Rudolf Jörres (CPC-M) und Prof. Marcus Mall (TLRC). In December the LID hosted the forum “Living with COPD. DZL experts Prof. Holger Schulz (CPC-M), Prof. Tobias Welte (BREATH) und Prof. Klaus F. Rabe (ARCN) were on hand to present, meet with patients and answer their questions. Attendance was close to 100 participants. For patients not able to get to the seminars the sessions were available online.

The LID also had information booths at a number of events in 2013. Notably were the Annual Meeting of the German Society for Pneumology (Deutsche Gesellschaft für Pneumologie und Beatmungsmedizin, DGP) as well as “Symposium Lunge” which, with 2700 participants, is the largest event in Germany for patients with lung disease. The LID also actively interacts with patient organizations to receive feedback and suggestions for topics to cover. The LID is staffed by two science editors and receives support from many scientists, clinicians, and members of the DZL.

DZL International Symposium 2013

Second DZL International Symposium in Munich

The Second DZL International Symposium took place on October 4 and 5 at the Leonardo Royal Hotel in Munich in conjunction with the 3rd Munich Lung Conference. The conference focused on 'Lung Aging: Molecular Mechanisms and Clinical Relevance'. The conference was organized by the Institute of Lung Biology and Disease / Comprehensive Pneumology Center (CPC) and was financially supported by the HMGU, the DZL e.V., as well as by the DZL Munich site and the Helmholtz 'Lung Biology and Disease' Research School.

The topic of the conference struck a chord with researchers. As the first international conference addressing aging mechanisms of the lung, it was well-attended with close to 200 participants from the national and international lung research communities and was rated as very innovative and informative by all parties. Even though there is a lot of circumstantial evidence for the impact of aging on chronic lung disease, world-wide research on aging in lung disease is still underrepresented.

The conference featured 29 oral presentations of internationally well-known speakers and chosen scientists and nearly 90 posters on topics relevant to lung aging and beyond. The DZL awarded three junior scientists poster prizes. Many participants continued the scientific conversations at the conference's Bavarian Evening in an Oktoberfest-ive shack atmosphere and used this opportunity to renew existing networks and to build new ones. The meeting was used for internal DZL networking as well – DZL scientists used the opportunity to hold DZL Disease Area and Platform Meetings.

The German Centers for Health Research

The main objective of the German government's framework program for health research is to more effectively combat complex common diseases that are becoming increasingly prevalent in the population. To create favorable conditions for achieving this goal, the Federal Ministry of Education and Research has established the German Centers for Health Research. These Centers have been set up as long-term, equal partnerships between non-university research institutions and universities with university hospitals.

The German Centers for Health Research leverage existing competencies and thus make a significant contribution to closing knowledge gaps and to improving prevention, diagnosis and treatment. The aim is to achieve the highest possible level of therapeutic efficacy for each patient. The Centers' research policy emphasizes close cooperation between the basic and clinical research units of all partners, oriented on the indications and the needs of the patients. The close networking and expansion of existing research structures enable a faster transfer of research findings into clinical practice (translational research).

Over the long term, the strategic collaboration of leading scientists in the German Centers for Health Research will make Germany internationally more competitive as a science location and markedly more attractive for young researchers both within Germany and from around the world.

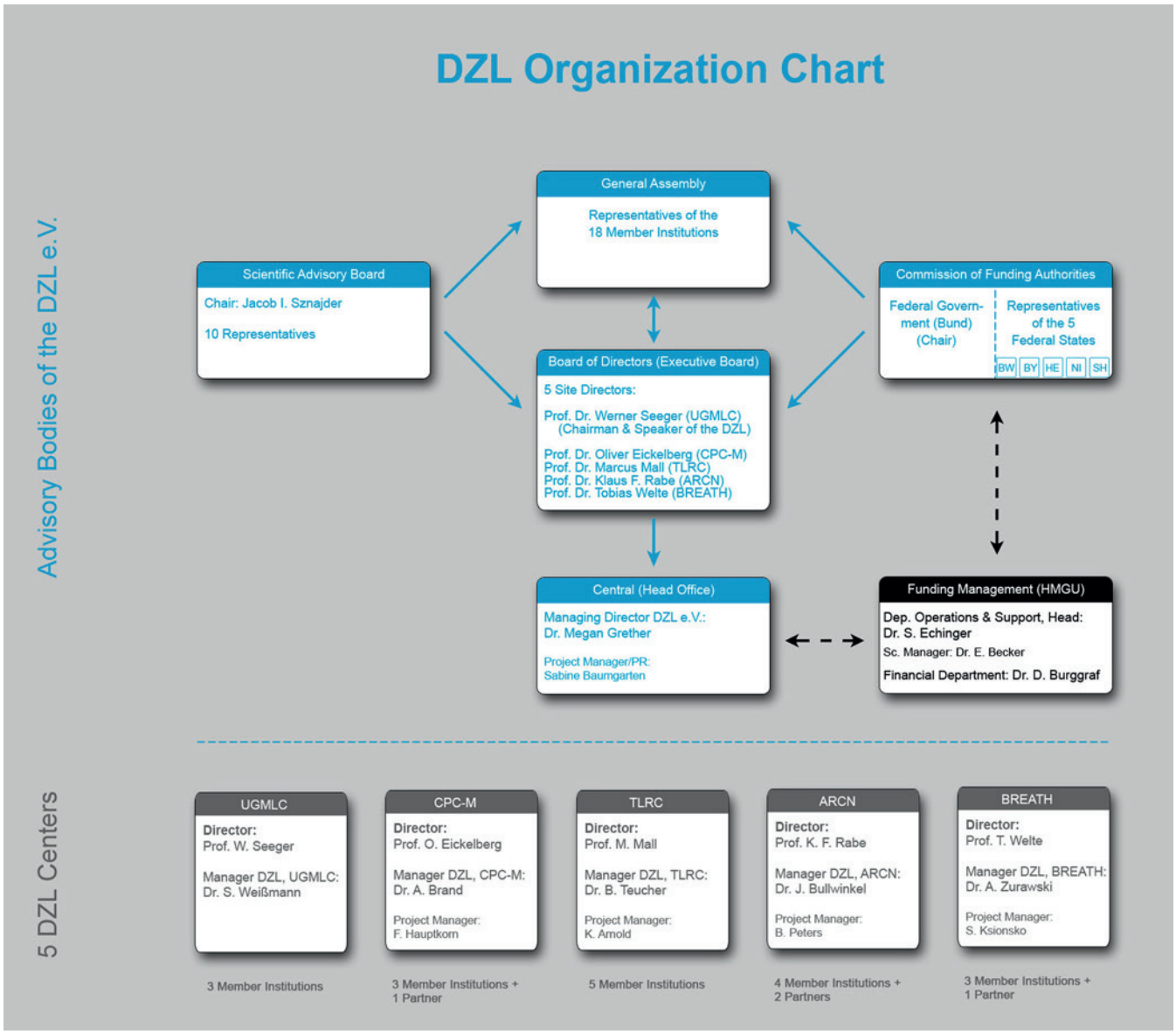
In 2009 the German Center for Neurodegenerative Diseases (DZNE) and the German Center for Diabetes Research (DZD) were founded. In 2011 four additional German Centers for Health Research were established: the German Center for Infection Research (DZIF), the German Center for Cardiovascular Research (DZHK), the German Consortium for Translational Cancer Research (DKTK) and the German Center for Lung Research (DZL). A steering committee in which all partners participate coordinates the joint research activities as well as the division of tasks and use of resources for all sites of the respective center, in accordance with the jointly defined research priorities.

DZL Organization

In the DZL 200 principal investigators and their research groups work together to combat lung disease. These scientists are located at 22 top research institutes throughout Germany, and their activities are managed by five coordinating centers.

The DZL is overseen by a five member Board of Directors, and an International Advisory Board provides input on scientific strategy. The DZL General Assembly and Commission of Funding Authorities are also important governing bodies of the DZL. The scientific and administrative activities of the DZL are coordinated by the Central Head Office in Giessen in conjunction with the Local Managers. Financial administration is managed by the Funding Management Office based at the Helmholtz Zentrum München.

DZL Organization Chart



DZL Head Office

The Head Office of the German Center for Lung Research is located at Justus-Liebig University in Giessen. It supports the work of the Board, is responsible for the coordination and implementation of DZL programs, coordinates internal and external communications, and is the point of contact for all matters relating to the DZL. The Managers of the five sites work in close collaboration with the DZL Head Office to support and coordinate the work of the DZL.

Chairman and Speaker of the DZL, Director of the UGMLC site: Prof. Dr. Werner Seeger

Managing Director:

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Tel.: +49 (0)641 99-46724

Project Coordinator/Public Relations:

Sabine Baumgarten
sabine.baumgarten@ugmlc.de
Tel.: +49 (0)641 99-46721

DZL Board of Directors

The DZL is led by a five member Board of Directors, one from each DZL partner site. The Chairman and Speaker of the DZL Board is Prof. Dr. Werner Seeger of the UGMLC site. The other Board members are Prof. Dr. Oliver Eickelberg (CPC-M), Prof. Dr. Marcus A. Mall (TLRC), Prof. Dr. Klaus F. Rabe (ARCN), and Prof. Dr. Tobias Welte (BREATH).

Funding Management Office

The Funding Management office (FMM) is located at the Helmholtz Zentrum München. FMM is responsible for providing coordination, development and advice to the DZL on matters relating to finance and grant legislation. Funds are allocated to the DZL by FMM in accordance to regulations and decisions from the BMBF. DZL scientists are supported and advised by FMM with respect to grants and budget management and management reports. Tasks include examination of applications for fund release (AZA) and handling of enquiries related to the best use of resources in line with funding regulations. The FMM team is required to provide to the BMBF cash outflow statements, the results of the compliance testing, as well as the economic and investment plans of the DZL. Currently, FMM is an interdisciplinary team of 5 employees from the fields

DZL Board of Directors. From L to R: O. Eickelberg, T. Welte, K. Rabe, W. Seeger, M. Mall

DZL Managers and Head Office. From L to R: B. Teucher, A. Zurawski, J. Bullwinkel, M. Grether, S. Weissmann, A. Brand, S. Baumgarten

of finance (Dr. Dorothe Burggraf, Claudia Fricke, Katrin-Alexandra Pickl) and science (Dr. Stefan Echinger, Dr. Eva Becker).

Commission of the Funding Authorities

The Commission of Funding Authorities (Kommission der Zuwendungsgeber, KdZ) oversees the DZL's cooperation with the DZL granting agencies. 90% of the DZL funds are provided by the Federal Ministry for Education and Research (Bundesministerium für Bildung und Forschung, BMBF) with the remaining 10% coming from the respective states (Länder) in which a funded institution is located. The BMBF and the states each send a representative to the KdZ, which is chaired by the representative of the federal government. States contributing funds to the DZL are Baden-Württemberg, Bavaria, Hesse, Lower Saxony, and Schleswig-Holstein.

DZL General Assembly

The General Assembly (Mitgliederversammlung) is the central decision-making body of DZL. It is composed of representatives from each of the 18 DZL member institutions. The General Assembly elects the members of the Board of Directors and the Board Chair. On the basis of proposals from the DZL Board of Directors, the General Assembly decides on issues of fundamental importance to the DZL including determination of scientific priorities, allocation of responsibilities amongst the sites, and the proposed use of resources in accordance with reviewer recommendations.

Bundesministerium
für Bildung
und Forschung

Baden-Württemberg
MINISTERIUM FÜR WISSENSCHAFT,
FORSCHUNG UND KUNST

Niedersächsisches Ministerium
für Wissenschaft und Kultur

Bayerisches Staatsministerium für
Bildung und Kultus, Wissenschaft und Kunst

Ministerium für Bildung
und Wissenschaft
des Landes Schleswig-Holstein

HESSEN
Hessisches Ministerium
für Wissenschaft und Kunst

DZL Scientific Advisory Board

The DZL is honored to have a distinguished board of internationally renowned experts as advisors.

Professor Peter J Barnes
Head of Respiratory Medicine, Imperial College London

Professor Rachel Chambers
Professor of Respiratory Cell and Molecular Biology, Center for Respiratory Research, University College London

Jeffrey M Drazen, MD
Distinguished Parker B. Francis Professor of Medicine, Harvard Medical School; Editor-in-Chief, New England Journal of Medicine

Professor Stuart Elborn
Professor of Respiratory Medicine, Director CF Center, Belfast City Hospital, President ECFS, Centre for Infection and Immunity, Queen's University Belfast

Mark Gladwin, MD
Division Chief, Pulmonary, Allergy, and Critical Care Medicine, Director Vascular Medicine Institute, University of Pittsburgh Medical Center

Marlene Rabinovitch, MD
Professor of Pediatric Cardiology, Stanford University School of Medicine

Susan Shurin, MD
Deputy Director, National Heart, Lung, and Blood Institute (NHLBI), National Institutes of Health (NIH)

Stephen G Spiro, MD
Honorary Physician, University College London Hospitals and The Royal Brompton Hospital

Peter M Suter, MD
Akademien der Wissenschaften Schweiz, Centre Medical Universitaire, University of Geneva

Jacob I Sznajder, MD
Chief, Division of Medicine-Pulmonary, Ernest S. Bazley Professor of Asthma and Related Disorders, Northwestern University Feinberg School of Medicine

DZL Coordinating Centers

HANNOVER

BIOMEDICAL RESEARCH IN ENDSTAGE AND OBSTRUCTIVE LUNG DISEASE HANNOVER (BREATH)

Medizinische Hochschule Hannover

Leibniz Universität Hannover

Fraunhofer-Institut für Toxikologie und Experimentelle Medizin in Hannover

CAPNETZ Stiftung

DIRECTOR

Prof. Dr. Tobias Welte

HEIDELBERG

TRANSLATIONAL LUNG RESEARCH CENTER (TLRC)

Universitätsklinikum Heidelberg

Ruprecht-Karls-Universität Heidelberg

Thoraxklinik am Universitätsklinikum Heidelberg

Deutsches Krebsforschungszentrum (DKFZ)

European Molecular Biology Laboratory (EMBL)

DIRECTOR

Prof. Dr. Marcus A. Mall

BORSTEL/LÜBECK/KIEL/GROßHANSDORF

AIRWAY RESEARCH CENTER NORTH (ARCN)

Forschungszentrum Borstel

Universität zu Lübeck

Universitätsklinikum Schleswig-Holstein – Campus Lübeck

Universitätsklinikum Schleswig-Holstein – Campus Kiel

Christian-Albrechts-Universität zu Kiel

LungenClinic Grosshansdorf

DIRECTOR

Prof. Dr. Klaus F. Rabe

GIEßEN/MARBURG/BAD NAUHEIM

UNIVERSITIES OF GIESSEN AND MARBURG LUNG CENTER (UGMLC)

Justus-Liebig-Universität Gießen

Philipps-Universität Marburg

Max-Planck-Institut für Herz- und Lungenforschung in Bad Nauheim

DIRECTOR

Prof. Dr. Werner Seeger

Speaker and Chairman of the DZL

MUNICH

COMPREHENSIVE PNEUMOLOGY CENTER MUNICH (CPC-M)

Helmholtz Zentrum München – Deutsches Forschungszentrum für Gesundheit und Umwelt

Ludwig-Maximilians-Universität München

Klinikum der Universität München

Asklepios Fachkliniken München-Gauting

DIRECTOR

Prof. Dr. Oliver Eickelberg

Associated partners are shown in grey

Airway Research Center North (ARCN)

Borstel, Lübeck, Kiel, Großhansdorf

- Research Center Borstel
- University of Lübeck
- University Clinic Schleswig-Holstein, Lübeck Campus
- University Clinic Schleswig-Holstein, Kiel Campus
- Kiel University
- LungenClinic Grosshansdorf

Prof. Dr. Klaus F. Rabe

- Director of ARCN
- Medical Director of the LungenClinic Grosshansdorf
- Professor of Pneumology, Kiel University
- Chairman of the Institute for Lung Research (ILF)
- President of the European Respiratory Society (ERS) 2011/2012

Contact

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Number of DZL Principal Investigators: 28

Research Profile

Scientists and clinicians of the Airway Research Center North (ARCN) focus on research on chronic obstructive pulmonary disease (COPD), lung cancer as well as asthma and allergy. This translational research consortium combines top level expertise in basic research and medicine in the field of pulmonology in Schleswig-Holstein. Together with its partners in the DZL, ARCN aims to find more effective ways to prevent disease, to provide earlier diagnoses, and to develop enhanced, individualized therapies for patients with lung disease. In keeping with the approach of the DZL, ARCN researchers pursue a holistic approach to study of the lung, including studying disease pathogenesis, the progression of inflammatory and proliferative processes, and the regeneration and/or repair of diseased lung tissue.

As the biggest North-German clinic specialized in lung and airway diseases with more than 13,000 patients treated per year, LungenClinic Grosshansdorf, together with the University Clinic Schleswig-Holstein (UKSH) and the Medical Clinic Borstel, is responsible for clinical and patient-oriented research in ARCN. The Research Center Borstel is devoted to investigation of infectious as well as non-infectious lung diseases and is key to the success of ARCN basic research and animal models. Additional partners are researchers at the University of Lübeck and the Christian-Albrechts-University Kiel. These scientists test asthma in animal models, analyze the epigenetic background of lung diseases and develop novel imaging techniques.

To strengthen the connection between clinical and basic research, the Biomaterialbank Nord has been installed as central infrastructure. This crosslink between complementary partners in ARCN is intended to support the collaborative implementation of translational research strategies.

Biomedical Research in Endstage and Obstructive Lung Disease Hannover (BREATH)

Hannover

- Hannover Medical School (MHH)
- The Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM)
- Leibniz Universität Hannover
- CAPNETZ Stiftung

Prof. Dr. Tobias Welte

- Director of BREATH
- Chairman of the German Sepsis Society
- Speaker for the Clinical Study Center Hannover (KS-MHH; set up by the BMBF)
- Member of the Presidium of the German Interdisciplinary Association for Intensive Care and Emergency Medicine (DIVI)
- Chairman of the Board of Trustees of the CAPNETZ Stiftung
- Head of the Competence Center for Infectious Diseases
- Director of the Competence Network ASCONET
- President of the German Society of Pneumology

Contact

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Number of DZL Principal Investigators: 46

Research Profile

In the BREATH research network, doctors and scientists from Hannover Medical School (MHH), the Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM), the Center for Health Economics Research Hannover (CHERH) of the Leibniz Universität Hannover (LUH), and the CAPNETZ Stiftung have come together to carry out research in the field of lung diseases with the aim of optimizing the care structure for patients, including gaining new knowledge, developing and expanding current therapeutic measures, stemming and reducing mortality in this field and generally improving the quality of life of patients with lung diseases. There is also close cooperation with the REBIRTH Cluster of Excellence. A major focus of BREATH is clinical research, particularly in the fields of lung transplantation and stem cell therapy. In 2012 at the Hannover Medical School, DZL scientists from BREATH were involved in the first living lung donation in Germany.

The Department of Respiratory Medicine at MHH is engaged in the lung transplantation program and conducts research in the fields of infectious disease, allergic disease, and pulmonary hypertension. Basic research on infectious diseases focuses on inflammatory cells in the pulmonary system and on proteolytic enzymes in connection with infection. In cooperation with Fraunhofer ITEM, research scientists investigate the pathophysiology of allergic diseases and have access to the cutting edge pollen exposure room at ITEM. Researchers at LUH bring significant expertise in the fields of health services and health economics to the DZL. Finally, the nation-wide research network, CAPNETZ (Network of Excellence Community Acquired Pneumonia), is now integrated into the DZL. CAPNETZ connects clinical, microbiological and basic research in order to gain knowledge about the pathogenesis of community acquired pneumonia (CAP), a significant public health challenge. CAPNETZ is the most comprehensive CAP database in the world.

Comprehensive Pneumology Center Munich (CPC-M)

Munich

- Helmholtz Zentrum München – German Center for Environmental Health
- Ludwig Maximilian University Munich
- Munich University Hospital
- Asklepios Clinic Munich-Gauting

Prof. Dr. Oliver Eickelberg

- Director of CPC-M
- Chairman of the Comprehensive Pneumology Center
- Director of the Institute of Lung Biology and Disease, Helmholtz Zentrum München
- Professor of Experimental Pneumology at Ludwig-Maximilian University Munich

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Number of DZL Principal Investigators: 37

Research Profile

At the Comprehensive Pneumology Center Munich (CPC-M), the Helmholtz Zentrum München, Ludwig Maximilian University Munich with its University Hospital and the Asklepios Clinic Munich-Gauting come together to form one of the largest centers for translational research on chronic lung disease world-wide. The Helmholtz Zentrum München is a renowned expert in bridging fundamental research and applied medical research with a strong focus on translational medicine in the area of lung disease. Ludwig Maximilian University is one of the top level universities in the German Excellence Initiative and its medical faculty is involved in high level pulmonary research and medical care. The Asklepios Clinic Munich-Gauting is one of the leading hospitals in Germany that specializes in lung diseases.

Research at CPC-M is focused on chronic lung diseases. CPC-M scientists integrate state-of-the-art techniques in molecular and (stem) cell biology, pharmacology, molecular pathology and clinical medicine in order to develop new diagnostic tools and therapies. CPC-M scientists are coordinators for the Disease Areas “Diffuse parenchymal Lung Disease” and “Asthma and Allergy”.

As an important link between clinical and experimental research the CPC-M operates the CPC outpatient unit where researchers and clinicians work close together to interlink scientific results and therapeutic approaches. In addition to its research program the CPC-M coordinates the German-French Lung School together with the CPC Research School and Graduate Program “Lung Biology and Disease”. The CPC-M also operates the Lung Information Service (www.lungeninformationsdienst.de) which is responsible for effective public and patient education and outreach about lung diseases.

Translational Lung Research Center (TLRC)

Heidelberg

- Heidelberg University Hospital
- Ruprecht-Karls-University, Heidelberg
- Thoraxklinik at Heidelberg University Hospital
- German Cancer Research Center (DKFZ)
- European Molecular Biology Laboratory (EMBL)

Prof. Dr. Marcus A. Mall

- Director of TLRC
- Chairman of the Translational Lung Research Center
- Director of the Department of Translational Pulmonology
- Head of the Division of Pediatric Pulmonology & Allergy and Cystic Fibrosis Center

Contact

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Number of DZL Principal Investigators: 34

Research Profile

The Heidelberg Translational Lung Research Center (TLRC) is an interdisciplinary center for translational lung research in which physicians and scientists at the Heidelberg University Hospital and Medical Faculty of Heidelberg, the Thorax Clinic at the Heidelberg University Hospital (one of Germany's largest hospitals specialized on lung diseases), the German Center for Cancer Research, and the European Molecular Biology Laboratory work together to combat lung disease. Our common goal is to improve diagnosis and therapy of chronic lung diseases in children and adults by promoting the close collaboration and exchange of expertise between basic research and clinical science.

The research focus is on elucidating the mechanisms underlying common genetic and acquired chronic and malignant lung diseases such as cystic fibrosis, COPD, and lung cancer. The scientists' goal is to identify new therapeutic targets to improve early diagnosis and develop more curative treatment options. Within the basic research program cell- and animal models are used to investigate molecular causes of chronic airway diseases with a focus on the role of the airway epithelium. We make use of next generation-sequencing, as well as state-of-the-art immunology and molecular biology techniques. Results from these experiments will improve our understanding of airway mucus obstruction and chronic inflammation in cystic fibrosis and other chronic obstructive lung diseases, such as COPD and asthma. Systems biology is applied to improve our understanding of the molecular causes of lung cancer. Early clinical trials are conducted to make new diagnostic and therapeutic strategies available to patients in a timely manner.

Universities of Giessen and Marburg Lung Center (UGMLC)

Giessen, Marburg, Bad Nauheim

- Justus-Liebig University Giessen
- Philipps University Marburg
- Max Planck Institute for Heart and Lung Research in Bad Nauheim

Prof. Dr. Werner Seeger

- Chairman and Speaker of the German Center for Lung Research (National Cnter)
- Director of UGMLC
- Managing Director of the Department for Internal Medicine, Justus Liebig University Giessen
- Director, Department of Lung Development and Remodeling, Max Planck Institute for Heart and Lung Research
- Director of the Excellence Cluster “Cardio-Pulmonary System” (ECCPS)

Contact

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Number of DZL Principal Investigators: 55

Research Profile, UGMLC

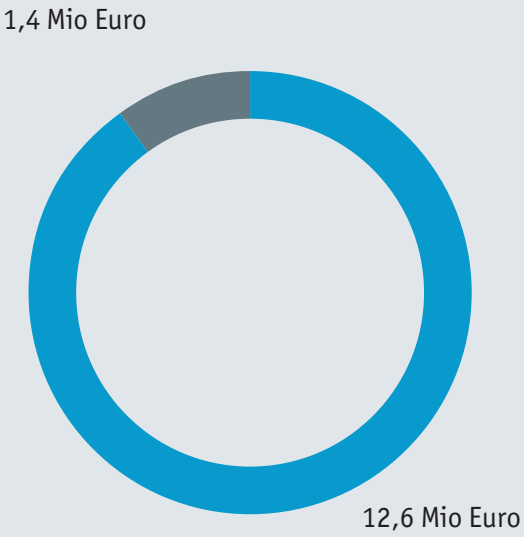
Translational research at the Universities of Giessen and Marburg Lung Center (UGMLC) deals with lung diseases caused by inflammatory and hyperproliferative processes. This includes research on the impact of environmental factors on the development of asthma as well as on the development and therapy of Chronic Obstructive Pulmonary Disease (COPD), with special focus on the alterations of airways and blood vessels. In the Disease Area Pneumonia and Acute Lung Injury (ALI), UGMLC concentrates on the role of innate immunity and inflammatory mechanisms in the acute disease and during resolution and regeneration. Molecular and cellular mechanisms that may help developing efficient regenerative therapies are studied in the Disease Areas Lung Fibrosis (DPLD) and Pulmonary Hypertension (PH). The UGMLC partners complement one another by a close interplay of basic research and clinical research, which is based on the cooperation of the Max-Planck-Institute, the universities and the university hospital. Marburg focuses on the areas of asthma and COPD, Giessen on DPLD and PH, where Giessen can be regarded as a national and international center. The Max-Planck-Institute in Bad Nauheim complements the clinical and translational science with basic research in the fields of stem cell research, developmental biology and cell signaling pathways. Further synergies result from cooperation with the other DZL sites as well as other networks (such as Asconet and Cosyconet) and local research consortia like the Cluster of Excellence Cardio-Pulmonary System (ECCPS).

Within the DZL, UGMLC hosts the DZL Central Office and the DZL Biobank and Data Management Platform.

Prizes

Awarded to	Prize
Prof. Dr. Oliver Eickelberg CPC-M, Helmholtz Zentrum München, Munich University Hospital	Gay-Lussac Humboldt Research Prize 2013
Prof. Dr. Andreas Günther (shared prize) UGMLC, Justus Liebig University Giessen	Oskar Medicine Prize 2013
Dr. Soni Savai Pullamsetti UGMLC, Max Planck Institute for Heart and Lung Research, Bad Nauheim	Young Investigator Award, 5th World Symposium on Pulmonary Hypertension, Nice, France
Prof. Dr. Gesine Hansen (shared prize) BREATH, Hannover Medical School	Eva Luise Köhler Research Prize for Rare Diseases
Prof. Dr. Didier Stainier UGMLC, Max Plank Institute for Heart and Lung Research, Bad Nauheim	Officier in l'Ordre de Léopold de Belgique
Dr. Frauke Stanke BREATH, Hannover Medical School	Adolf Windorfer Prize
Prof. Dr. Erika von Mutius CPC-M, Munich University Hospital	Gottfried Wilhelm Leibniz Prize 2013

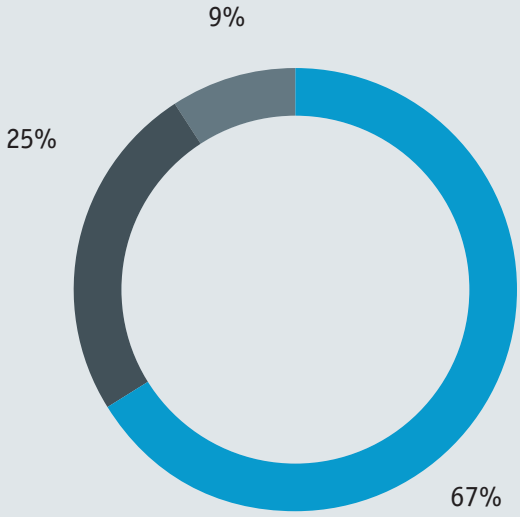
Financials and Personnel



Total Funding

The total funding for the DZL in 2013 was 14 Million Euro. 90% was received from the Federal government and 10% from the five German states with participating DZL Centers. The Funding Management Office at the Helmholtz Zentrum München distributed the project funding to the respective partner institutions. Across the eight disease areas studied by DZL scientists more than 50 major research projects are addressed.

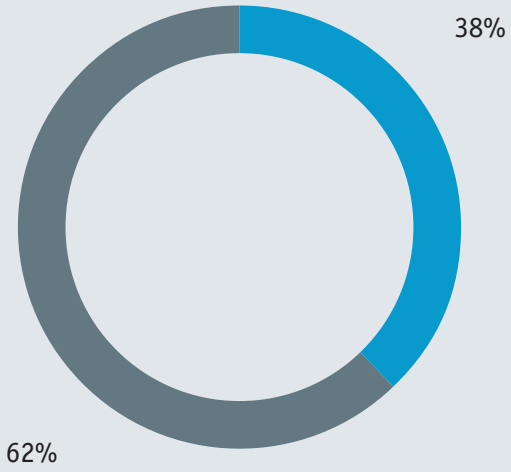
- Federal Government
- States (Baden-Württemberg, Bavaria, Hesse, Lower Saxony, Schleswig-Holstein)



Cost Breakdown – DZL 2013 Expenses

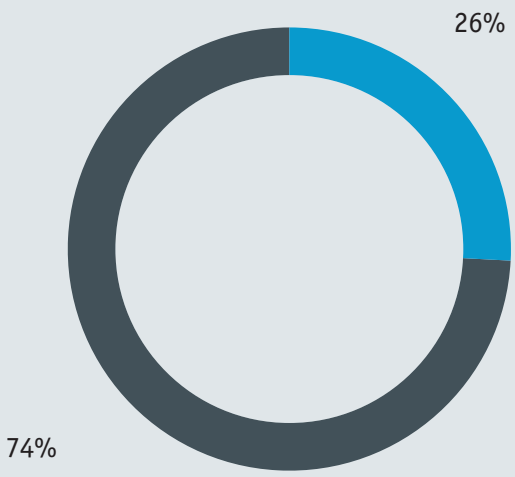
- Personnel
- Consumables
- Equipment

Cost Breakdown – DZL e.V. Expenses



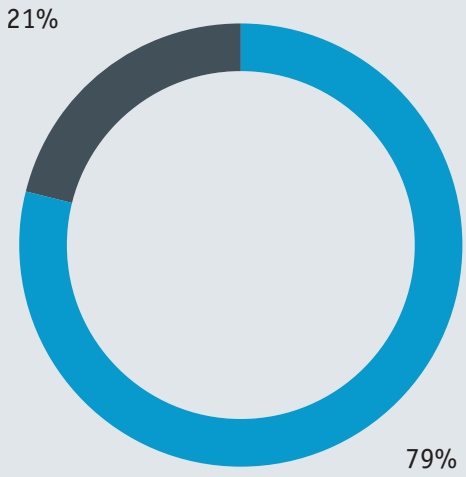
The DZL e.V. is financed through membership fees from each partner institution, amounting to €325,000 in 2013. The 2013 Annual Financial Statement and Year-end Close of the DZL e.V. was conducted by the firm Haas & Haas.

- Personnel
- Consumables



DZL-Funded Personnel

- Male
- Female



DZL Principal Investigators

- Male
- Female

Personnel and Equal Opportunities

In 2013 251 employees received with DZL funds across the five partner centers, an increase of 84 when compared to 2012. Of the 251 funded employees, 129 were scientists and 122 support staff. 74% of these employees were women, an increase of 4% over 2012.

There are 200 affiliated principal investigators (PIs) in the DZL, although not all of them receive DZL funds. In 2013 a concerted effort was made to increase the number of women on the DZL faculty. Of the 32 PIs who became DZL members in 2013, 14 of them were women, bringing the overall percentage of women PIs in the DZL up to 21%, an increase of 5% relative to 2012.

Patents

DZL Scientists are actively involved in translating research discoveries into inventions. Below is a selection of published patents involving DZL scientists with a priority date of 2011 or later.

DZL Center	Priority Date	Applicant (Inventor)	Title and Publication Number(s)
CPC-M	2012	PLS-Design GmbH, Klinikum rechs der Isar, TU München, Helmholtz Zentrum München (Reinhard Bredehorst, Thomas Grunwald, Markus Ollert, Carsten Schmidt-Weber, Edzard Spillner)	Selective Local Inhibition of TNFR1-mediated Functions at the Site of Antigen/Allergen Presentation EP000002746396A1 US020140178474A1
CPC-M	2012	PLS-Design GmbH, Klinikum rechs der Isar, TU München, Helmholtz Zentrum München (Reinhard Bredehorst, Thomas Grunwald, Markus Ollert, Carsten Schmidt-Weber, Edzard Spillner)	Controlled activation of complement components for use as endogenous adjuvant EP000002674167A1 US020130337045A1
CPC-M	2012	PLS-Design GmbH, Klinikum rechs der Isar, TU München, Helmholtz Zentrum München (Reinhard Bredehorst, Thomas Grunwald, Markus Ollert, Carsten Schmidt-Weber, Edzard Spillner)	Modulation of effector T cell responses by local depletion of complement component C3 EP000002674168A1 US020130337044A1
CPC-M	2012	F. Hoffmann LaRoche GmbH, Ludwig-Maximilians-Universität München (Carole Bourquin, Rafaella Castoldi, Stefan Endres, Christian Klein, Sebastian Kobold, Gerhard Niederfellner, Claudio Sustmann)	Bispecific antibody molecules with antigen-transfeceted T-cells and their use in medicine WO0020131136l5A1
TLRC	2012	Ruprechts-Karl-Universität Heidelberg (Marcus A. Mall, Raman Agrawal, Martina Muckenthaler)	Micro-RNA-148b in Pathogenesis and as New Therapeutic Target in Chronic Obstructive Pulmonary Diseases (COPD an CF) WO2013/174692

DZL Center	Priority Date	Applicant (Inventor)	Title and Publication Number(s)
UGMLC	2012	Carsten Kirschning, Stefan Bauer, Hubertus Hochrein, Bavarian Nordic A/S (Carsten Kirschning, Hubertus Hochrein)	Agonists and antagonists of toll-like receptor (TLR) 13 WO0020131173348A1
UGMLC	2012	Max-Planck-Gesellschaft Zur Förderung Der Wissenschaften E.V. (Stefan Offermanns, Boris Strilic, Dagmar Schumacher, Nina Wettschureck)	Novel Therapeutic target for the prevention of tumour metastases EP000002641969A1 WO0020131399940A1
ARCN	2011	Med Laserzentrum Luebeck GmbH, Thorlabs GmbH, Univ zu Lubeck (Christian Lührs, Dierck Hillmann, Peter Koch, Alfred Vogel, Gereon Hüttmann)	Method for Optical Tomography EP127176022 US 201161508831 P
BREATH	2011	Medizinische Hochschule Hannover (Ulrich Martin, Christina Mauritz)	Novel Method for the Production of Differentiated Respiratory Epithelial Cells AU002012213402A1 EP000002670841A1 US020140017691A1
BREATH	2011	Medizinische Hochschule Hannover (Axel Haverich, Mathias Wilhelmi, Thomas Aper)	Method and Device for Producing a Bioartifical Tissue Construct WO0020130918654A1
BREATH	2011	Medizinische Hochschule Hannover (Axel Haverich, Mathias Wilhelmi, Thomas Aper)	Method for Producing a Biological Tissue Construct and Use of Specifically Obtained Autologous Cells DE102011112955A1 WO0020130373349A1

DZL Center	Priority Date	Applicant (Inventor)	Title and Publication Number(s)
CPC-M	2011	Ludwig-Maximilians-Universität München, Universität Basel, Schweizerisches Tropen- und Public Health Institut, Universität für Bodenkultur Wien (Erika von Mutius, Charlotte Braun-Fahrländer, Wolfgang Kneifel)	Raw milk preparation for use for preventing or treatment of Asthma and other allergic diseases in infants and children EP000002548457A1 WO002013011040A1
UGMLC	2011	Activaero GmbH (Tobias Gessler, Thomas Schmehl, Werner Seeger, Robert Voswinckel)	Administration of iloprost as an aerosol bolus AU002012247562A1
UGMLC	2011	Indiana University Research And Technology Corporation (Matthias Clauss, Irina Petrache, Robert Voswinckel)	Monoclonal antibody and antigens for diagnosing and treating lung disease and injury WO002012170929A3

Masthead

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German Center for Lung Research (DZL e.V.)

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