Ludwig Boltzmann Institute
Lung Vascular Research

ANNUAL REPORT

2012

Content

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Ludwig Boltzmann Institute for Lung Vascular Research

1. The Institute in Overview

The Ludwig Boltzmann Institute for Lung Vascular Research (LBI-LVR) is a non-profit research institute founded in July 2010 by the Ludwig Boltzmann Gesellschaft (LBG) - an Austrian non-university research organisation who acts as carrier institution for Ludwig Boltzmann Institutes doing research in the field of Human Medicine/ Life Sciences and in the field of Humanities. The LBI for Lung Vascular Research was established after a demanding two-level evaluation by international peers who strongly recommended its founding.

The LBI-LVR, like the other Ludwig Boltzmann Institutes, is based on a partnership between organisations and institutes that traditionally carry out research and organisations that traditionally apply research. The LBI-LVR Consortium, comprising the Ludwig Boltzmann Gesellschaft as carrier institution in partnership with the Medical University of Graz, Bayer HealthCare, Nebu-Tec and, until 2012, the Austrian Academy of Sciences, brings together excellent researchers and business partners into a single, multi-disciplinary and translational institute.

Director of the LBI-LVR is Univ. Prof. DDr. Andrea Olschewski who supervises the whole research team that consists of four research groups reflecting the four programme lines of the institute (programme lines A-D). The Advisory Board of the LBI is composed from the representatives of the partner organisations (LBG, MUG, Bayer Austria, Nebu-Tec and, until 2012, the OEAW). Besides monitoring the progress of the LBI, this panel allows the partners to make propositions, decide together and commission the director of the LBI with the subsequent implementation. The Scientific Advisory Board of the LBI is an independent world-wide recognised group of experts in pulmonary vascular biology, in pulmonary hypertension and in radiological diagnostic of pulmonary vascular diseases. It monitors the scientific activities of the LBI.

The budget of the newly established institute is app. 8.9 million Euro for the first four years. The Ludwig Boltzmann Society covers 55% of the total costs. The remaining 45% of the costs are shared by the consortium of our partners.

The institute is located at the Center for Medical Research (ZMF), Stiftingtalstrasse 24, 8010 Graz, Austria. If you like to contact us, please call **+43 (0)316-385-72057** or send us an email to: **office@lvr.lbg.ac.at**. You can also visit our website: **http://lvr.lbg.ac.at**

1.1 What you should know about Lung Vascular Diseases: Facts, Diagnostics, and Therapy

Lung vascular diseases have emerged as a leading field of research. Over the past 10 years, the diagnostics and therapy of the prototype disease pulmonary hypertension have made tremendous progress. Pulmonary hypertension (PH) is a chronic, prolonged crippling and fatal disease, and it is notoriously under diagnosed – usually it has progressed to a late stage before it is diagnosed. Epidemiological data is not available for the entire field of pulmonary hypertension; however, approximately 15% of all patients suffering from the most common causes of death worldwide, severe heart failure and chronic obstructive lung disease, develop PH which has a major impact on survival.

Progressive loss of exercise capacity and suffering from dyspnoea represent the most threatening features for individuals affected by this disease. Clinical care for PH is currently extremely costly and prolonged, therefore, this condition poses a large burden on the Austrian as well as on the European healthcare system. Current therapies improve exercise capacity and may prolong survival of the affected individuals, but are far away from curing the disease or providing a substantially prolonged lifespan or a good quality of life.

The Ludwig Boltzmann Institute for Lung Vascular Research aims to provide a significant contribution to early recognition of pulmonary vascular diseases including pulmonary hypertension via novel and non-invasive methods. It further aims to develop new pathology-based therapies and to prevent disease progression to the stage where highly costly therapies are necessary, thus prolonging life expectancy and improving health and quality of life.

The integrative, multidisciplinary and translational structure of the LBI-LVR allows it to uncover underlying molecular pathways, identify distinct targets for reverse-remodelling therapy, foster drug development based on these targets, and prove these new treatment options in preclinical and clinical pilot trials.

1.2 Mission Statement / Aims of the Institute

The LBI for Lung Vascular Research represents one of the worldwide leading research centres for pulmonary hypertension. Its expertise in the investigation of basic mechanisms of pulmonary vascular constriction and remodelling, combined with a broad and profound clinical background, allows the development of improved standards for patient therapy. Our aim is to develop and implement new diagnostic tools for non-invasive diagnosis and innovative therapeutic strategies, thereby securing significant improvements in diagnostics of pulmonary vascular diseases, treatment and therapy, an improved prognosis and better quality of life for the victims of this serious disease. All our actions, both in research and implementation, will be based on mutual respect and esteem with regard to the patients, to our partners, and our staff.

In summary, the main objectives of the LBI for Lung Vascular Research are:

- Exploring mechanisms of pulmonary vascular disease to allow the identification of both significant new therapeutic targets and new disease biomarkers that could enable specific diagnosis and therapy monitoring (programme lines A-D)
- Improving pulmonary and intrapulmonary selectivity of vasoactive drugs or compounds for reverse remodelling (programme line A-B).
- Establishing animal models with clinically relevant end points (programme line B).
- Developing new diagnostic tools for non-invasive screening for pulmonary vascular diseases (programme line C).
- Implementing the achieved results into preclinical as well as clinical pilot studies (programme line D).
- Increasing awareness for pulmonary vascular diseases in the society and for healthcare providers (programme line D)

1.3 Partners of the Institute

We thank our partners, all members of the Ludwig Boltzmann Society and the managing committee as well as the Government of Austria for their continuous support.

Ludwig Boltzmann Society (http://www.lbg.ac.at/en)

The Ludwig Boltzmann Gesellschaft (LBG), our most important partner, is a private non-profit sponsor of research establishments in Austria. It is named after the Austrian physicist, mathematician and philosopher Ludwig Boltzmann, whose broad scientific interests still remain the basis for the interdisciplinarity of the Ludwig Boltzmann Gesellschaft today. The LBG, which is financed from public and private resources, is divided into institutes and clusters and currently employs more than 250 people.

Medical University of Graz (http://www.medunigraz.at/)

The Medical University of Graz (MUG) has declared strategically to enhance research on vascular diseases ("kardiovaskuläres Forschungsfeld" or "focus on cardio-vascular research") giving highest priority for this field of interest. Research activities of the MUG cover a broad range of clinical as well as pre-clinical fields. The MUG applies a pro-active approach in research management to involve and integrate researchers in international research initiatives. The LBI for Lung Vascular Research fits excellently in this concept and will be an important core for promotion of lung vascular research, diagnostic and innovative therapy of lung vascular diseases at the MUG.

Bayer Health Care (http://www.bayerhealthcare.com/scripts/pages/en/)

Pulmonary hypertension is one of the main focus-points of Bayer Health Care (BHC). BHC is currently developing new therapeutic options for the treatment of pulmonary hypertension, like the soluble guanylate cyclase stimulator Riociguat. BHC has a broad experience in pulmonary hypertension associated research and in the transfer of results from "bench to bedside". The interest of BHC is to further understand the underlying pathophysiology and associated pathological changes and conditions in PH.

Nebu-Tec (http://www.nebu-tec.de/)

Nebu-Tec GmbH was established with the primary focus to develop and manufacture inhalation devices for the treatment of pulmonary hypertension. In addition to clinical and patient treatment, Nebu-Tec devices are used by different biotechnology companies in

research. The strategic interest of Nebu-Tec to participate in the LBI is to further develop a relevant and realistic market of intrapulmonary delivery of targeted PH drugs via specialised inhalation devices.

Austrian Academy of Sciences (http://www.oeaw.ac.at/) until 2012

The Austrian Academy of Sciences (OEAW) is the leading non-university research institution in Austria. It is the core-scope of the OEAW to participate in national and international scientific research programmes. The OEAW was represented by the Institute of Biophysics and Nanosystems Research (IBN) until 2012. In 2012, the IBN was transferred to the Karl Franzens University Graz, the Graz University of Technology, and the Medical University of Graz. The partnership with the LBI is continued with the researchers who are now employed by the Medical University of Graz. This research group under the supervision of Prof. Ruth Prassl extends existing knowledge and expertise in regard to the development of lipid-based pulmonary delivery systems.

1.4 Advisory Board and Scientific Advisory Board

Advisory Board of the Partners (Board)

Mag. Claudia Lingner, Ludwig Boltzmann Gesellschaft

Prof. Dr. Dr. h.c. Irmgard Theresia Lippe, Medical University of Graz

PD Dr. Hubert Trübel, Bayer Health Care

Stefan Kern, Nebu-Tec

Scientific Advisory Board (SAB)

Prof. Sally-Ann Cryan – Royal College of Surgeons, Dublin, Ireland

Prof. Steve Abman – University of Colorado, US

Prof. Wolfgang Kuebler – University of Toronto, Canada
Heart Institute of Berlin, Germany

Prof. Jose Lopez-Barneo – University of Sevilla, Spain

Prof. Dean Sheppard – University of California, US

The Scientific Advisory Board Meeting was held on September 28th, 2012 in Vienna.

1.5. Personal and human resources development

Staff of the LBI

The LBI-LVR staff consists of the director, the programme line leaders, senior and junior researchers (PhD students) and master students, technicians, study nurses and administrative assistants.

In the description PL-A,B,C and D indicate programme lines A, B, C and D, respectively.

Name	PL	entry	Function
Avian Alexander, Mag.Dr.	D	9/11	Statistician. Affiliation: Institute for Medical Informatics, Statistics, and Documentation at the Medical university of Graz. He supports mainly the PL-D in study design.
Bálint Zoltán, Dr.	C	9/10	Group leader of PL-C. His responsibilities include the planning and coordination of experiments performed by the programme line in the development of a quantitative, non-invasive and reproducible technique for hemodynamic assessment of the pulmonary circulation by means of chest computed tomography imaging.
Biasin Valentina, MSc.	A1	10/10	Junior scientist in PL-A and PhD student of the Doctoral programme Molecular Medicine at the MUG. Supervised by Dr. Grazyna Kwapiszewska & Dr. Andrea Olschewski & Dr. Robert Eferl (LBI for Cancer Research). Her primary task is to decipher novel candidate genes involved in PH development.
Blümel Peter, MSc.	B1	1/11 – 8/12	Technician in PL-B supporting the research activities of Dr. Marsh' group.
Chandran Nagaraj, MSc	A2	4/11	Junior scientist in PL-A and PhD student of the Doctoral programme Molecular Medicine at the MUG. He is supervised by Dr. Andrea Olschewski & Dr. Horst Olschewski. His research focus is the regulation of ion channel function in human pulmonary arteries.
Crnkovic Slaven, MSc	A2	10/11	Junior scientist in PL-A and PhD student of the Doctoral programme Molecular Medicine at the MUG. His research focus is the vascular remodelling in chronic hypoxia-induced pulmonary hypertension.

Egemnazarov Bakytbek, Dr.	A1	11/11	Post-Doc. His research focus is the characterisation of right heart hypertrophy.
Fazakas Csilla, MSc	C	1/12	Junior scientist in PL-C. She does cell culture and experiments with pulmonary arterial endothelial cells. Furthermore, she performs measurements of the transendothelial electric resistance, endothelial permeability, PCR screening, western blot, and immunostaining for junctional changes.
Foris Vasile, Dr. med. univ.	D	10/10	Junior scientist in PL-C and PhD student of the Doctoral programme Molecular Medicine at the MUG. He focuses on PH biomarkers and is supervised by Dr. Gabor Kovacs & Dr. Andrea Olschewski & Dr. Horst Olschewski.
Ghanim Bahil, Dr. med. univ.	D	10/11	Junior scientist in PL-D and physician at the Medical University of Vienna. Affiliation: Department of Thoracic Surgery at the Medical university of Vienna. He supports the institute in human tissue sampling.
Halsegger Sabine	A and B		Associated technician as a member of the Experimental Anaesthesiology. Affiliation: Department of Anaesthesiology at the Medical University of Graz. She supports the research activities of the Experimental Anaesthesiology.
Helmberger Michael	C	4/12	Master student in PL-C. His duty is to develop an automatic algorithm for lung vessel detection from chest computed tomography images.
Hoffmann Julia, Dr.	A1	03/12	Post-Doc. She investigates lung vessel remodelling in animal models and humans with pulmonary hypertension. Her focus is the examination of the signal pathways via microarrays.
Hrzenjak Andelko, Ass. Prof. Dr.	A	9/10	Senior scientist in PL-A and assistant professor in the Department of Pulmonology at the MUG. He supports the institute in the field of proteomics.
Jain Pritesh, MSc	B2	10/10	Junior scientist in PL-B and PhD student of the Doctoral Programme Molecular Medicine at the Medical University of Graz. He is currently testing and analysing liposome formulations. Supervised by Dr. Ruth Prassl & Dr. Leigh Marsh & Dr. Andrea Olschewski.
Jakob-Pelikan Claudia	all	12/12	LBI-LVR Assistant of the Director. Responsible for administrative tasks.

Kovacs Gabor, Dr.med. univ.	D	10/10	Group leader of PL-D.
Külper-Siefken Sigrid	all	5/11-1/13	LBI-LVR Assistant of the Director. Responsible for administrative tasks.
Kwapiszewska Grazyna, Dr.	A A1	11/10	Deputy Director of the LBI-LVR and group leader of PL-A.
Leber Regina, Dr.	B2	9/10	Senior scientist in PL-B and responsible for the development of stable drug nanocarriers for controlled released pulmonary therapy. She performs and coordinates experiments in regard to drug-carrier formulations, nebulisation of drug carriers, as well as the in vitro and in vivo test systems.
Leithner Katharina, Dr. med. univ.			Associated junior scientist and PhD student of the Doctoral Programme Molecular Medicine at the MUG. Supervised by Dr. Horst Olschewski & Dr. Andrea Olschewski & Prof. Berthold Huppertz. Affiliation: Department of Pulmonology at the Medical University of Graz. She focuses on hypoxia-dependent pathways in non-small cell lung cancer.
Mandl Anna-Maria	all	8/10 – 2/13	LBI-LVR Assistant of the Director.
Marsh Leigh, Dr.	B1	1/11	Group leader of PL-B.
Nagler Lisa Maria, BSc	D	7/11 - 11/12	Study nurse in PL-D who supports the clinical study group of the LBI.
Nagy Bence, MSc	A		Associated junior scientist in PL-A and PhD student of the Doctoral programme Molecular Medicine at the MUG. Supervised by Dr. Andrea Olschewski & Dr. Horst Olschewski & Dr. Gerd Kostner. Affiliation: Department of Pulmonology at the Medical University of Graz. He focuses on novel pathways of pulmonary hypertension.
Niklasson Ida, MSc	A1	11/11	Technician in PL-A who supports the research activities in the group of Dr. Kwapiszewska.
Olschewski Andrea, Prof. DDr.	A2	7/10	Director of the LBI.
Olschewski Horst, Prof. Dr.	D	7/10	Head of the Department for Pulmonology at the MUG. He supports PL-D in planning clinical studies and coordination with the other programme lines of the LBI.

Papp Rita, PhD	A	9/12	Post-Doc. She investigates the vasoconstrictive remodelling in the lung vessels including the examination of ion channels via molecularbiological and electrophysiological methods e.g. patch-clamp.
Pienn Michael, DI	C	10/10	Junior scientist in PL-C and PhD student at the Technical University of Graz. His focus is dynamic assessment of pulmonary circulation and computed tomography image analysis. He is supervised by Dr. Zoltán Bálint and Prof. Rudolf Stollberger.
Prassl Ruth, Prof. Dr.	B2	7/10	Supporting scientist of the LBI in PL-B and principal investigator at the Institute of Biophysics and Nanosystems Research (IBN) of the Austrian Academy of Sciences (OEAW). Her scientific contribution focuses on the development of nanoparticulate aerosol drug formulations.
Reinisch Sabrina, BSc	B	7/11	Technician in PL-B supporting the research activities of the group of Dr. Marsh.
Schittl Julia	A	12/10	Technician in PL-A supporting the research activities in Dr. Kwapiszewska group.
Schloffer, Maria	A and B		Associated technician as a member of the Experimental Anaesthesiology. Affiliation: Department of Anaesthesiology at the Medical University of Graz. She supports the research activities of the Experimental Anaesthesiology.
Schratter Gebhard, BSc	B	10/11	Technician in PL-B supporting the research activities of the group and is responsible for the preparation of liposomal formulations.
Stacher-Priehse Elvira, Dr. med. univ.	A	9/10	Senior scientist in PL-A and pathologist at the Department of Pathology at the Medical University of Graz. She supports the institute in issues regarding human and animal lung histology.
Tscherner Maria, Dr. med. univ.	D	9/10	Junior scientist in PL-B and physician at the Department of Pulmology at the Medical University of Graz. She is responsible for invasive and non-invasive testing and their evaluation.
Wakonigg Gudrun, Mag. Dr.	all	3/12	PR Assistant of the Director.
Xu Hui, MSc	B1		Associated junior scientist in PL-B and PhD student of the Doctoral Programme Molecular Medicine at the MUG. Supervised by Dr. Leigh

			Marsh & Dr. Akos Heinemann & Dr. Andrea Olschewski & Dr Horst Olschewski. Affiliation: Department of Pulmonology at the Medical University of Graz. She is currently developing and analysing alternative inflammatory models of pulmonary hypertension.
Zabini Diana, MSc	A2	10/12 -	Junior scientist in PL-A and PhD student of the Doctoral Programme Molecular Medicine at the MUG supervised by Dr. Andrea Olschewski & Dr. Horst Olschewski & Dr. Akos Heinemann. Her research focus is vascular remodelling in chronic thromboembolic pulmonary hypertension.

Every PhD or postdoctoral fellow has an individual project and each group has scientific independence in generating their individual progress.

During the last year our institute has strongly grown. Due to this pleasant development, administrative duties have also grown immensely. Hence, an administrative restructuring of the institute management was necessary. Herewith, we like to introduce our new team to you: Stefanie Kainz is LBI-LVR assistant of the director, Claudia Jakob-Pelikan the LBI secretary and Gudrun Wakonigg the PR assistant of the director.

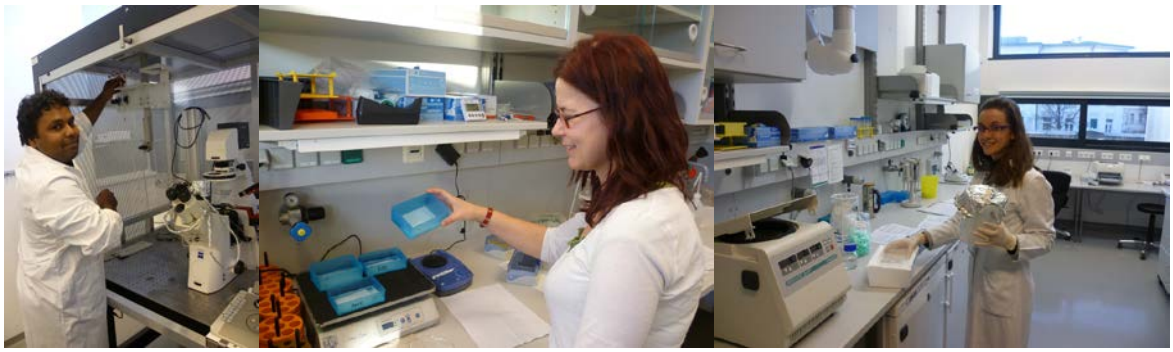
S. Kainz, C. Jakob-Pelikan, Director A. Olschewski, G. Wakonigg
(from left to right)

The following team members successfully joined advanced training courses in 2012:

Name	Date	Course
CRMKOVIC Slaven	DEC 2012	FELASA-B, Berlin, Germany
EGEMNAZAROV Bakytbek	DEC 2012	FELASA-B, Berlin, Germany
JAKOB-PELIKAN Claudia	NOV 2012	Modern office management – advanced course, Medical University Graz, Austria
MARSH Leigh	APR 2012	Embryo Transfer Course, Vienna, Austria
REINISCH Sabrina	SEPT 2012	FELASA B, Berlin, Germany
WAKONIGG Gudrun	SEPT 2012	Literature Search – The Basics of PubMed, Medical University Graz, Austria

1.6 Infrastructure

The LBI for Lung Vascular Research is predominately located at the Center for Medical Research (ZMF) at the MUG, which supports the LBI with cutting-edge research facilities guaranteeing high-yield development of this field. The aerosol studies are performed at the Institute of Biophysics at the MUG in Graz. The clinical research group of the PL-D is hosted by the Division of Pulmonology at the University Hospital of the MUG.



1.7. Highlights 2012

One of our main research foci is to establish a suitable method for a non-invasive and early assessment of pulmonary hypertension (PH). In 2012, **Dr. ZOLTÁN BÁLINT, Prof. Dr. HORST OLSCHEWSKI, Dr. GABOR KOVACS** and **DI MICHAEL PIENN** concentrated their investigations on the cardiac output which is an important diagnostic and prognostic factor in the haemodynamic evaluation of patients with PH. The gold standard for cardiac output measurement is thermodilution which requires an invasive right-heart catheterisation (RHC). The cardiac output determined with dynamic contrast-enhanced CT in the main pulmonary artery reliably predicted the values obtained by thermodilution during RHC. Moreover, from the same dynamic CT sequences the bolus propagation speed in the pulmonary artery correlated with the mean pulmonary artery pressure. Thus, this parameter can discriminate between patients with and without pulmonary hypertension. The easy determination of the propagation time and the comparably low dose for the examination make this measure suitable for everyday clinical practice and can provide a basis for PH diagnosis. This non-invasive technique might provide an alternative for repeated invasive right-heart catheter investigations in the follow-up of pulmonary arterial hypertension patients. Therefore, the first patent of our institute could be filed in 2012:

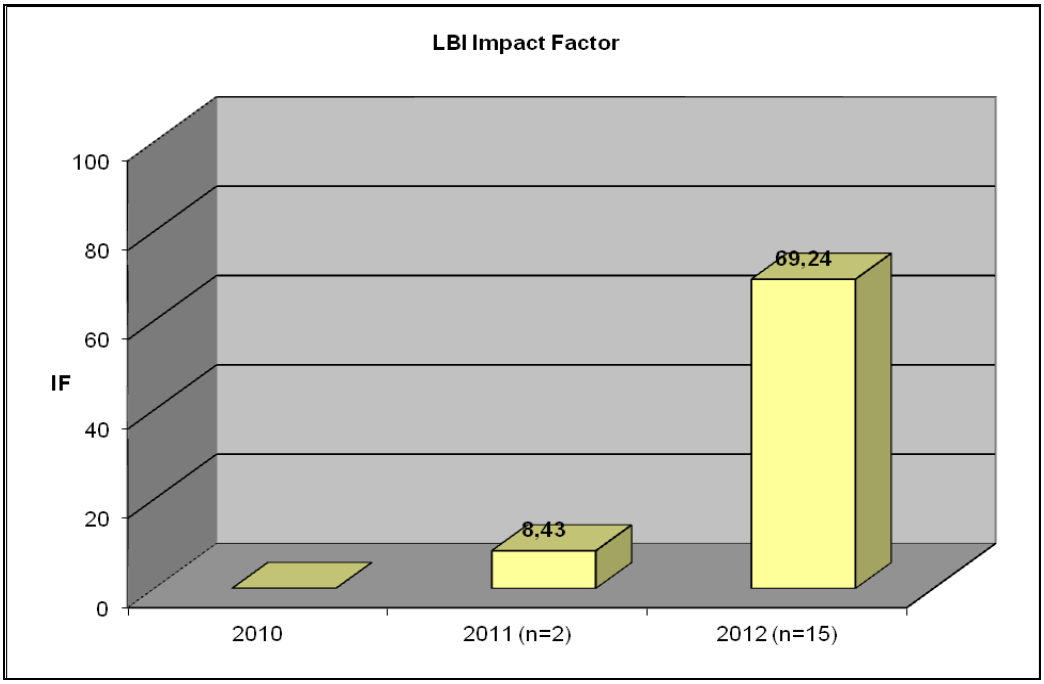
Pienn M, Kovacs G, Stollberger R, Olschewski H, Bálint Z. Methode zur nichtinvasiven Diagnose von pulmonaler Hypertonie. **Patent** File No. A 50258/2012.

Furthermore, 2012 was very successful for our institute. Our colleagues won several prizes: The Austrian Society for Pneumology (ÖPG) had announced three „**Short Term Fellowships**“ (EUR 5000.- each) provided by the companies Böhringer Ingelheim, AstraZeneca, and GlaxoSmithKline. The fellowships should enable the winners to learn a new scientific technique abroad and then establish it in Austria. Here our colleague **Dr. VASILE FORIS** successfully applied for this fellowship with the topic “Isolation and characterization of circulating endothelial cells and endothelial progenitor cells in pulmonary hypertension“. So he could visit the Descartes University Paris. In France, his supervisor Dr. David Smadja showed him how to isolate circulating mature endothelial cells.

The ÖPG also announced three **poster prizes** for clinical research, one awarded our colleague **Dr. MARIA TSCHERNER** who was then allowed to give a short presentation about her work: "Exercise induced increase of proANP in connective tissue disease patients may indicate PAP increase."

In March, our colleague **Dr. GABOR KOVACS** won the **Pfizer Young Researcher Award 2012** at the Pneumo Up2 Date Congress in Munich, Germany. He was honoured for his research paper: Kovacs G, Maier R, Aberer E, Brodmann M, Graninger W, Kqiku X, Scheidl S, Tröster N, Hesse C, Rubin L, Olschewski H. Pulmonary arterial hypertension therapy may be safe and effective in patients with systemic sclerosis and borderline pulmonary artery pressure. Arthritis Rheum. 2012, 64(4):1257-62. Furthermore, **Dr. GABOR KOVACS** could successfully make his **habilitation** in December 2012. The title of his talk was „Early diagnosis and early therapy of pulmonary hypertension in patients with scleroderma“.

Last but not least, our key researchers and their teams published a remarkable number of peer-reviewed papers in 2012. In the graphic, the impact factors of the last three years – since our institute was founded in 2010 - are shown. For this evaluation, all publications with LBI-LVR affiliation were counted. As you can see, the number of peer-reviewed papers and thus, the impact factor as indicator for the quality of our scientific work, significantly increased in 2012.



1.8 Public relations

The LBI for Lung Vascular Research has received several invitations to present the research work and aims of the institute.

A short overview about the activities is given here:

- Krebsmedikament begünstigt Lungenhochdruck - apotheke adhoc August 2012
- Krebsmedikament fördert Lungenhochdruck - derStandardat August 2012
- Krebs-Medikament kann zu Lungenhochdruck führen - APA August 2012
- Entschlüsselt: Zusammenhang Krebsmedikament und Lungenhochdruck - OTS August 2012
- Medikament zur gezielten Krebstherapie kann zu Lungenhochdruck führen - KleineZeitungat August 2012
- Pulmonale Hypertonie bei häufigen Lungenkrankheiten - Klinik Sonderausgabe Orphan Diseases June 2012
- LBI für Lungengefäßforschung - Steirer Monat Science, Das steirische Magazin für Wissenschaft und Forschung April 2012
- Feinstaubkonzentration, die der Raucher zu sich nimmt, ist exorbitant hoch – derStandardat March 2012
- Basic science in pulmonary vascular disease – Public Service Review October 2012

A highlight of our public relation activities is reflected in the work of our colleague Chandran Nagaraj who found out that the src tyrosine kinase is crucial for the potassium channel function in human pulmonary arteries. This finding leads to the displeasing assumption that if you inhibit the tyrosine kinases – as some modern cancer drugs do – this might result in pulmonary hypertension, a societal problem which will gain more and more importance in the close future. The mechanisms behind the relationship between these special anti-cancer therapies and pulmonary hypertension were described for the first time.

To improve the awareness of the society for lung diseases, and especially pulmonary hypertension, our institute closely collaborates with the "Austrian pulmonary hypertension self-help association" (www.lungen hochdruck.at/) in order to 1) Plan joint activities with patients, 2) Create central information points and 3) Provide comprehensive information for patients and relatives.

To meet patients and discuss the relevance of basic and clinical research is of great importance for clinicians and researchers, because it helps to keep our most important goal in mind: to ease symptoms of patients and to improve their prognosis.

The Long Night of Research (“Die lange Nacht der Forschung”) is the largest research and science event in Austria. Since 2005, the Austrian research institutions have the possibility to present their research to the broad public within one afternoon, evening and night. The LBI for Lung Vascular Research joined this event on April 27, 2012 for the first time. Visitors were able to go on a guided tour through the Division of Pulmonology, MUG/LKH Graz and through the ZMF building. Here molecularbiological techniques, such as Western blots and immunohistochemistry, were presented. Our colleagues Grazyna Kwapiszewska and Leigh Marsh introduced our guests to simple lab work such as preparing some solutions and handling the pipettes. In the pulmonology building, the visitors could observe some clinical methodologies, e.g. ECG.

Dr. Gabor Kovacs presents the Division of Pulmonology and performs together with Lisa Nagler a lung function test.

2. Research Programme 2012

The translational LBI-LVR combines experimental know-how, innovative imaging techniques and clinical background for high-yield research in an integrated approach. The research activities of the LBI-LVR are divided into four programme lines (PL A-D).

As the research projects of the institute are carried out on human and animal tissues and also include human studies, all projects are approved by the Ethical Committee or by the Austrian Federal Ministry for Science and Research (animal studies).

2.1. Programme line A: Grazyna Kwapiszewska: Pathomechanisms of Pulmonary Vascular Remodelling

Project Overview

Pulmonary vascular diseases have a multifactorial pathobiology, characterised by abnormalities of the smooth muscle, advential and endothelial cells which result in an obliterative remodelling of the pulmonary circulation, occlusion of the lumen in medium-sized and small pulmonary arteries due to excessive vasoconstriction, cellular proliferation and reduced apoptosis in the vascular wall and in situ thrombosis, as well as reduction in the number of pulmonary vessels. As a result, right ventricular afterload increases, leading to right heart failure and premature death. There has recently been a shift in treatment paradigm, from vasodilatory to anti-remodelling and even “reverse-remodelling” therapeutic

strategies, as new evidence suggests that the proliferative and antiapoptotic environment in the vascular wall of medium and small pulmonary arteries shares some features with neoplasia. Deciphering the molecular and cellular mechanisms underlying the pathobiology of pulmonary vascular remodelling, such as cytoskeletal reorganisation, dysregulation of cell growth and transcription factors and abnormal cell proliferation is the primary objective of our programme line. Besides, our second primary objective is the establishment of a human biobank that contains samples from IPAH, COPD and IPF as well as donor patient lungs as controls. More than 70 lungs have been collected so far and further samples will be collected in the next years.

Area A1, Grazyna Kwapiszewska: Novel signalling pathways contributing to the development of human pulmonary hypertension

Project Leader / Key Researcher
Name: Grazyna Kwapiszewska
Title: Dr. hum.biol.

Group Members
Biasin Valentina, Ph.D. student
Crnkovic Slaven, Ph.D. student
Egemnazarov Bakytbek, PostDoc
Hoffmann Julia, PostDoc
Kwapiszewska Grazyna, Project leader
Niklasson Ida, MTA
Schittl Julia, MTA
Stacher Elvira, Senior scientist

Research Results and Future Outlook

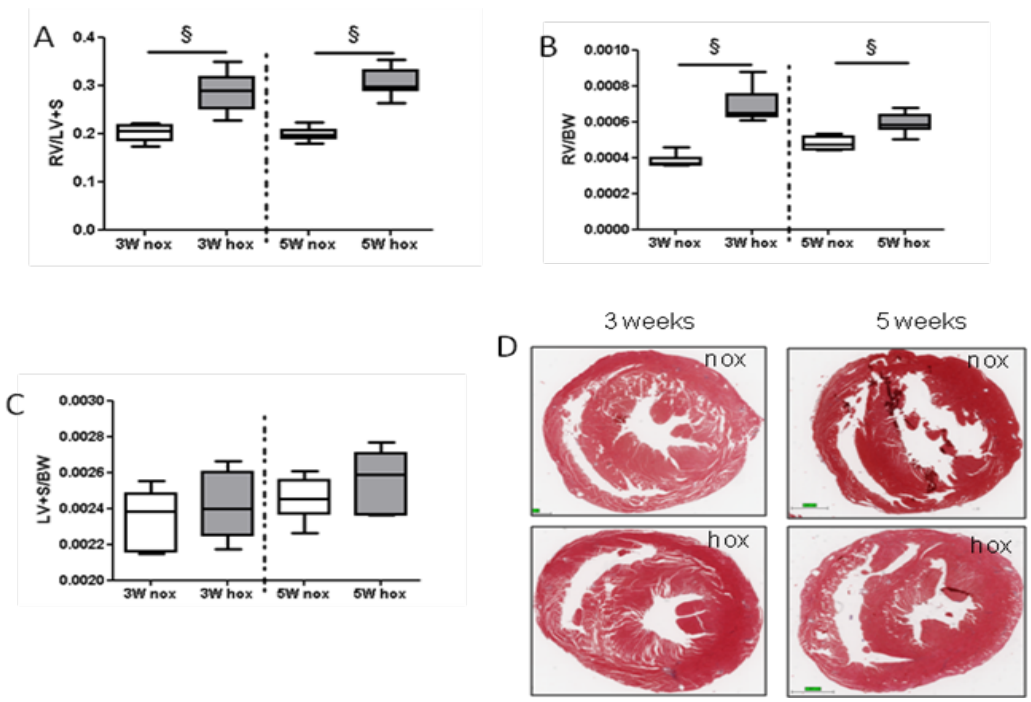
Continuation and optimisation of the human biobank

In 2011, the SOPs of lung collection in the biobank were established. In 2012, lung collection was continued and processes were optimised and refined in collaboration with Prof. Walter Klepetko, Medical University of Vienna, Austria. Large numbers of cryo-material, paraffin embedded tissue as well as smooth muscle cells, adventitil fibroblasts, and parenchymal fibroblasts have been collected and are now available for research. Microarray technology has been established from laser-microdissected vessels from patient groups versus donor patient lungs. In 2012, we have established the laser-microdissection of pulmonary vessels from patient groups versus donor patient lungs. Our pilot studies have verified that pre-

amplification of mRNA from laser-microdissected material delivers good quality and quantity which is satisfactory for application of microarray technology. So we have successfully established a human biobank with growing numbers of human samples. Therefore, in 2013 we aim to perform microarray experiments from human samples in order to find gene candidates that play an important role in vascular remodelling. Approx. 100 vessel profiles will be microdisected and pre-amplified to achieve enough material for our microarray studies. In the next step, we aim to focus on deciphering phenotypic differences between vessels from different human disorders. It will be performed by applying various molecular methods including immunohistochemical staining and real-time PCR.

<u>Deciphering the molecular pathways that lead to vascular remodelling and PH</u>

In 2012, we have established echocardiographic measurements in a mouse model of pulmonary hypertension. That is of great importance as echocardiography is a non-invasive method in PAH patients providing parameters of right ventricular function and dimension, which have diagnostic and prognostic value.



The figure shows the assessment of right and left ventricular histological changes in chronic hypoxia-exposed mice. Mice (n=4-8 per group) were exposed to normoxia or normobaric hypoxia (3 or 5 weeks). (A) Hypoxia-induced change in right ventricular to left ventricular and septum weight ratio (Fulton index). (B) Hypoxia-induced change in right ventricular to body weight ratio. (C) Effect of chronic hypoxia on left ventricular and septum to body weight ratio. (D) Representative photomicrographs of Masson's trichrome staining.

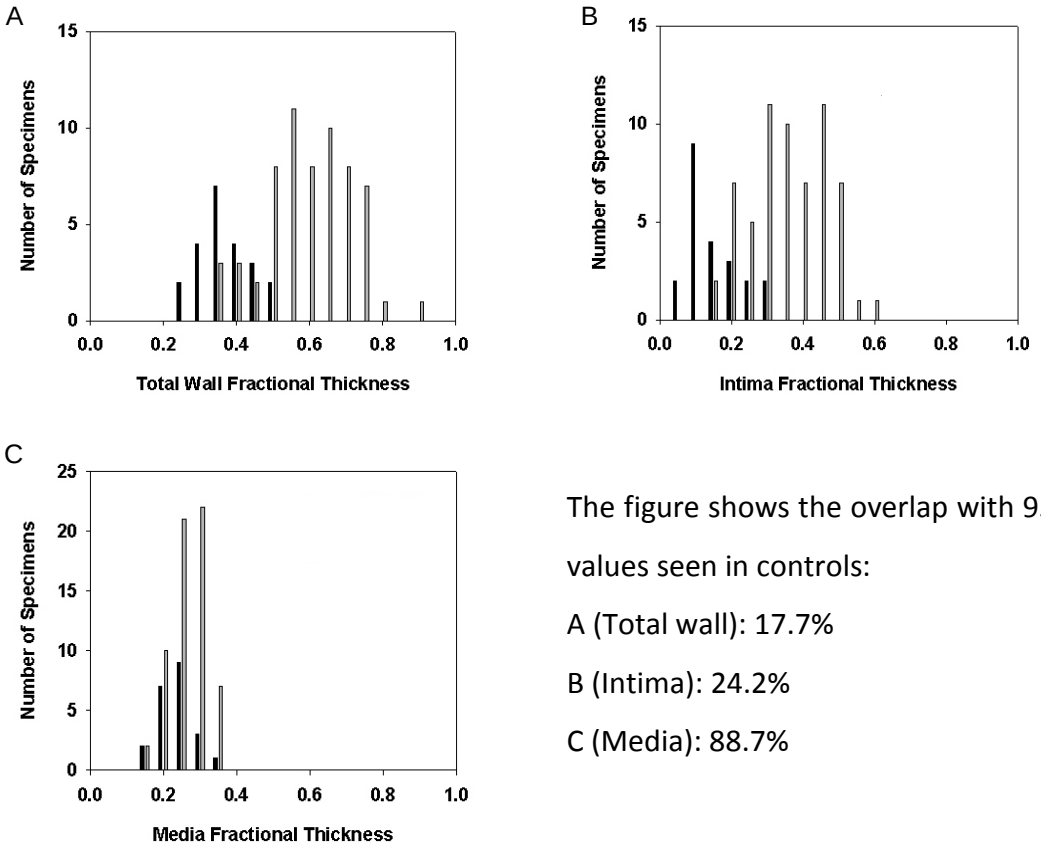
Consistent with changes described in patients with pulmonary hypertension, we observe in our model that RV free wall thickness was significantly increased and pulmonary artery acceleration time (PAAT) significantly shortened. Right ventricular end-diastolic diameter (RVEDD) and the diameter of the right ventricular outflow tract (RVOT) were increased, while the left ventricle (LV) showed a more elliptical geometry and increased eccentricity index indicative of RV dilatation and compression of the left ventricle. Hypoxia resulted in RV hypertrophy as demonstrated by an increased ratio of RV to LV plus septum weight (Figure A) or to body weight (Figure B). In comparison, no significant change in LV to body weight ratio was observed (Figure C). Masson's trichrome histological staining for collagen did not reveal any significant fibrotic changes in the right ventricle despite massive hypertrophy (Figure D, E).

Echocardiographic assessment of RV is difficult due to its position and shape. Therefore, functional significance of some EchoCG parameters used in clinics (e.g. TAPSE) is still under debate. Our lab, having established both EchoCG and RV pressure measurements using high fidelity pressure measurement system, has the unique opportunity to provide direct support for EchoCG parameters of the RV function with RV function parameters measured invasively.

„Modern age" pathology of pulmonary hypertension

This study was performed in Dr. Rubin Tuder´s lab in Aurora, Colorado, Colorado University by our colleague Dr. Stacher-Priehse. The histological findings of explanted lungs obtained from 62 PAH patients were compared with 28 control donor lungs in order to determine the spectrum of vascular pathology in the era of current PAH treatments. The results show that in the current era, medial hypertrophy no longer dominates in PAH-patients having been replaced by intimal hypertrophy. While statistically both, the media and the intima, are thickened, in the control lungs there is an overlap in terms of the fractional area thickness in 75% of the cases regarding the media compared to 24.5% regarding the intima. Plexiform lesions are a very common finding (90% of PAH lungs), whereas mutations in the BMPRII (bone morphogenetic protein receptor type II) are much less frequent than expected. Furthermore, inflammatory infiltrates are frequently observed in the PAH lung group. These infiltrates consist of mononuclear cells and their occurrence correlates with the intima-media thickness as well as the adventitial thickness.

Am J Respir Crit Care Med 2012; 186:261-72.



The figure shows the overlap with 95% range of values seen in controls:

A (Total wall): 17.7%

B (Intima): 24.2%

C (Media): 88.7%

Area A2, Andrea Olschewski: Function of ion channels and regulators of calcium homeostasis in pulmonary vasculature

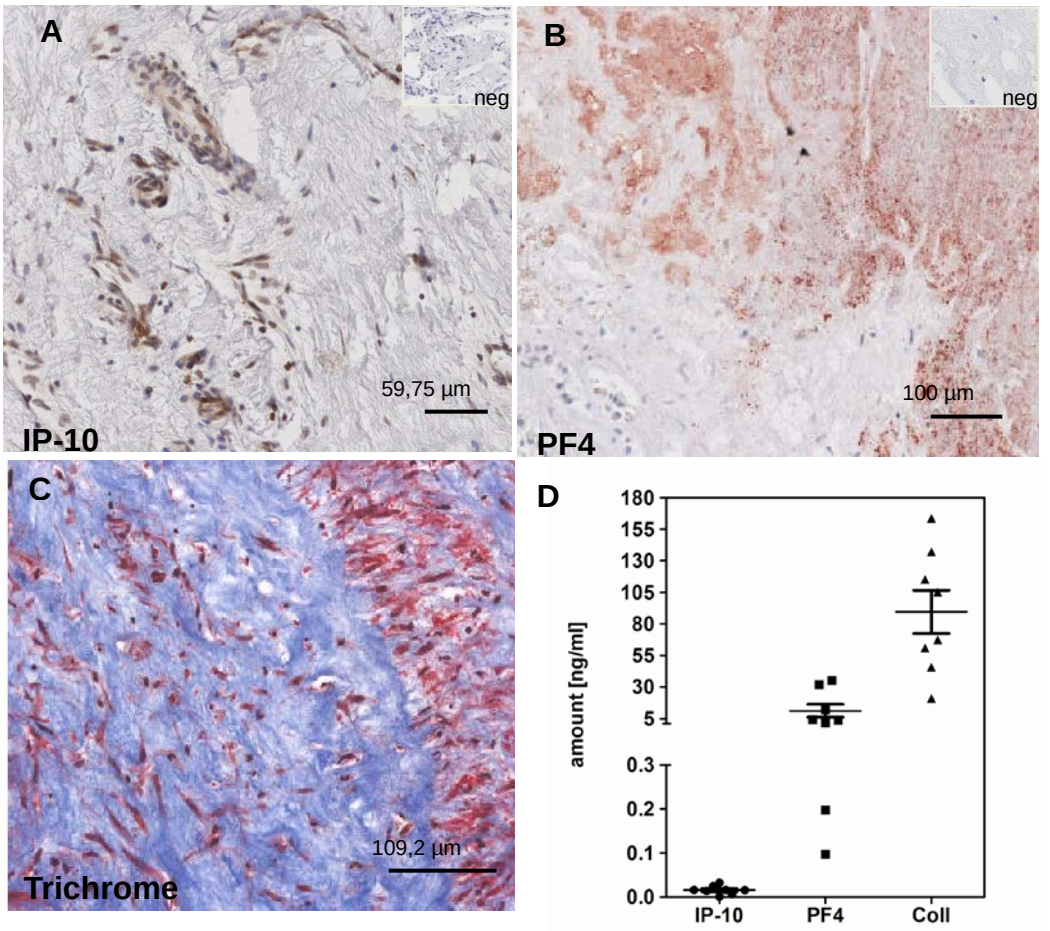
Project Leader / Key Researcher
Name: Andrea Olschewski
Title: Univ. Prof. Dr.

Group Members
Halsegger Sabine, Technician (associated)
Hrzenjak Andelko, Senior scientist
Nagaraj Chandran, Ph.D. student
Nagy Bence, Ph.D. student (associated)
Olschewski Andrea, Project leader
Papp Rita, PostDoc
Schloffer Maria, Technician (associated)
Zabini Diana, Ph.D. student

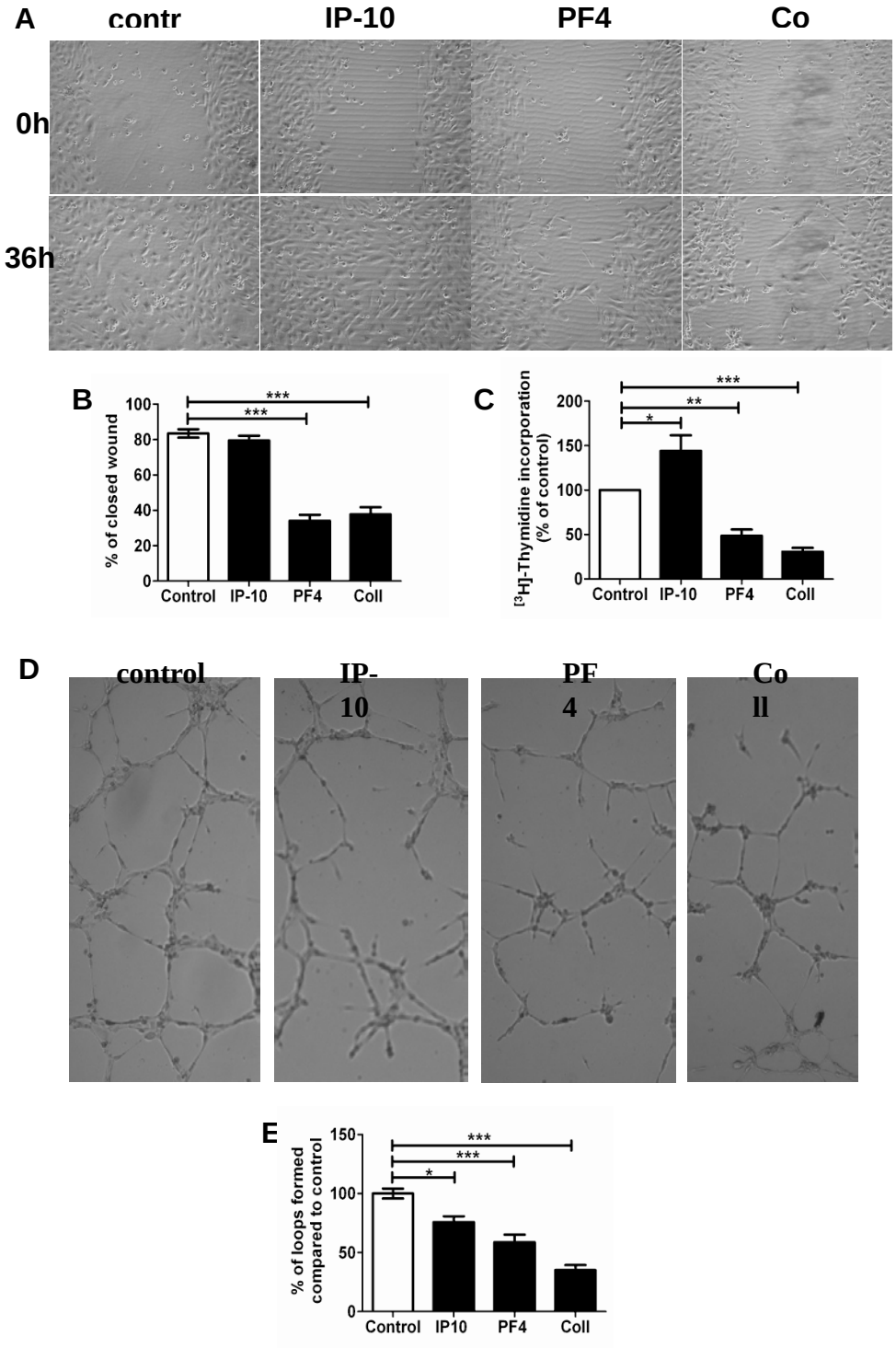
Research Results and Future Outlook

The role of endothelium in the angiogenesis in venous thromboembolism in the lung has been intensively investigated in 2012 in our group. We observed muscularized vessels and

non-muscularized vessels in the pulmonary endarterectomy (PEA) material, received as results of our collaboration with Prof. Walter Klepetko from the Medical University of Vienna. The isolated endothelial cells from the PEA material showed significantly different calcium homeostasis as compared to pulmonary artery endothelial cells (hPAECs) from normal controls. In the supernatant (FACS, ELISA) as well as on the tissue level (histochemical staining) of the PEA material, platelet factor 4 (PF4), collagen type I and interferon-gamma-inducible 10 kD protein (IP-10) were detected. CXCR3, the receptor for IP-10 and PF4, was particularly elevated in the distal parts of the PEA material as compared to human control lung (RT-PCR). PF4, collagen type I and IP-10 caused significant changes in calcium homeostasis and affected the cell proliferation, migration and vessel formation in hPAECs. The presence of angiostatic factors like PF4, collagen type I and IP-10 in the surgical PEA material from CTEPH patients may lead to changes in calcium homeostasis and endothelial dysfunction.



This figure illustrates immunohistochemical stainings for PF4 (A), trichrome staining (B), and IP-10 (C) present in PEA material. Negative controls are shown in the upper right insets. (D) Quantitative data of the amounts of IP-10, PF4 and collagen type I in the supernatant of PEA tissue were obtained from 8 different patients.



The figure shows the effect of IP-10, PF4 and collagen type I on the hPAEC function.
(A) Representative images of migration assay showing the effect of IP-10, PF4 and collagen type I on hPAECs. (B) Quantitative data of wound healing after 36h (number of experiments n=3). (C) Bar graph summarizing the effect of these factors on hPAEC proliferation compared to untreated hPAECs (n=3). (D) Representative image (4X) of vessels formed after 6h in Matrigel® under different conditions. (E) Bar graph representing the percentage of formed loops compared to control (n=3) (* $p<0.05$, ** $p<0.01$, *** $p<0.001$ compared to untreated cells).

Scientific Cooperations of A1 and A2

CIKES Nada Dr., University Zagreb, School of Medicine, Zagreb, Croatia

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KLEPETKO Walter Prof., Vienna Medical University, Austria

KRIZBAI István Dr., Institute of Biophysics, Biological Research Centre of the Hungarian Academy of Sciences, Szeged, Hungary

KULLNIG Peter Univ.-Doz. Dr., Diagnostikzentrum Graz für Computertomographie und Magnetresonanztomographie, Graz, Austria

LANG Irene Prof., Medical University of Vienna, Austria

LINDENMANN Jörg Dr., Division of Thoracic and Hyperbaric Surgery, Department of Surgery, Medical University of Graz, Austria

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Publications 2012 of A1 and A2

Papers with LBI affiliation

Fiorillo C, Moro F, Brisca G, Astrea G, Nesti C, Bálint Z, Olschewski A, Meschini MC, Guelly C, Auer-Grumbach M, Battini R, Pedemonte M, Romano A, Menchise V, Biancheri R, Santorelli FM, Bruno C. TRPV4 mutations in children with congenital distal spinal muscular atrophy. *Neurogenetics* 2012, 13(3):195-203. IF 3.488

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Li Y, Connolly M, Nagaraj C, Tang B, Bálint Z, Popper H, Smolle-Juettner FM, Lindenmann J, Kwapiszewska G, Aaronson PI, Wohlkoenig C, Leithner K, Olschewski H, Olschewski A. Peroxisome proliferator-activated receptor-β/δ, the acute signaling factor in prostacyclin-induced pulmonary vasodilation. *Am J Respir Cell Mol Biol*. 2012, 46(3):372-9. IF 5.125

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Books/ Book chapters

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Presentations at international conferences: oral communications

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Presentations at international conferences: posters

Crnkovic S, Hrzenjak A, Marsh LM, Olschewski A, Kwapiszewska G. Origin determination of remodeled parenchymal vessels in mouse pulmonary hypertension model using ephrinB2 and ephB4. Am J Respir Crit Care Med. 2012, 185 (A4759), San Francisco, US.

Marsh L, Zabini D, Xu H, Olschewski A, Kwapiszewska G: A potential role of HMGB1 in inflammatory vascular remodeling. 2nd Munich Lung Conference, Oct 2012, Munich, Germany.

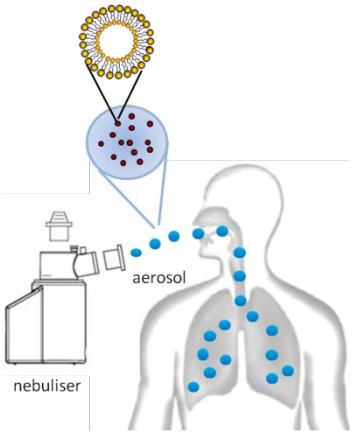
2.2. Programme line B, Leigh Marsh: Pharmacologic tailoring and assessment

Project Leader / Key Researcher
Name: Leigh Marsh
Title: Dr.

Group Members
Blümel Peter, Technician
Jain Pritesh, Ph.D. student
Leber Regina, Post-Doc
Marsh Leigh, Project leader
Prassl Ruth, Supporting scientist
Reinisch Sabrina, Technician
Schratter Gebhard, Technician
Xu Hui, Ph.D. student

Project Overview

Our programme line focuses on the development of enhanced therapeutic options for the treatment of pulmonary hypertension. Targeted drug delivery to the lung confers several advantages for the treatment of respiratory diseases. Pulmonary delivery can provide high drug concentrations locally while reducing potential systemic side-effects. By developing controlled released formulations we aim to improve the therapeutic efficacy by increasing drug half-life. The primary objective is to design, develop and characterise liposomes/micelles as nano-carriers for aerosol delivery to the lungs.

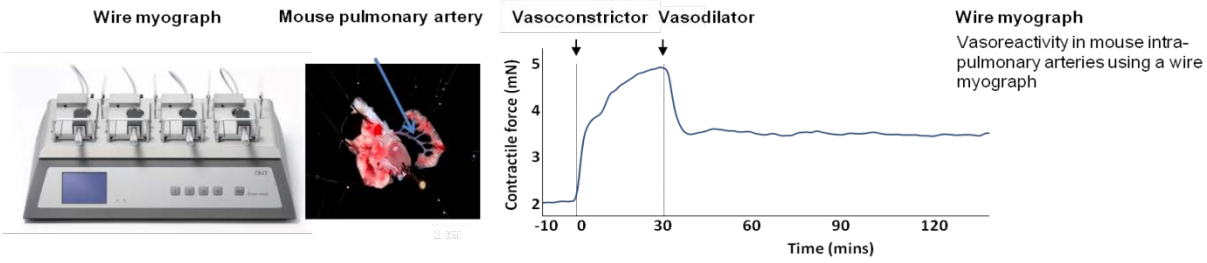


Within the second part of this project line we aim to utilise different experimental models of vascular remodelling and pulmonary hypertension to investigate the mechanisms underlying disease pathogenesis.

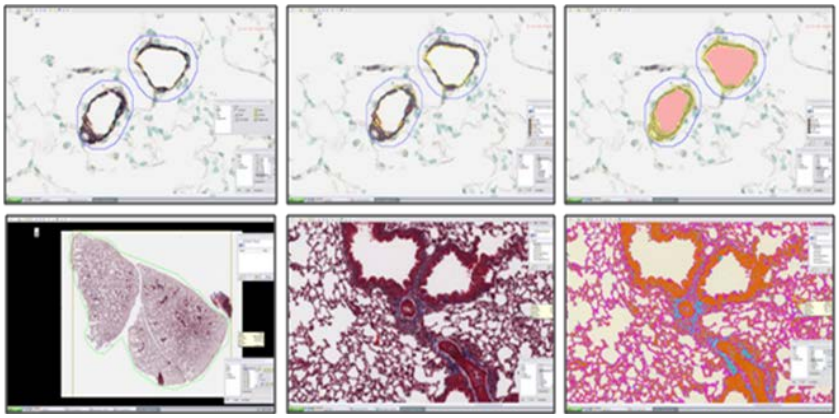
Research Results and Future Outlook

During 2012 we have developed and synthesised several new formulations based on liposome technology. These have been extensively characterised to determine their biochemical and biophysical properties. Parameters analysed include particles size, polydispersity index, zeta potential, drug encapsulation efficiency and long term stability in solution. The biological activity of the liposomal drug formulations in comparison to the free drug was determined by in vitro assays. The effect on cell viability was tested with two different cell lines, A549 and human primary pulmonary artery smooth muscle cells. None of the liposomal drug carriers exhibited any cytotoxic effects on proliferation rates.

Aerosol formation with liposomal emulsions was analysed with different nebulisers. The small aerosol droplet size produced allows an effective distribution of encapsulated drug in human lungs. Additionally, we have investigated the effects of mechanical stress generated during nebulisation on different liposomal complexes in terms of liposome stability, drug encapsulation and size distribution. The ex vivo wire myograph technique has been established using small intrapulmonary arteries from mice lungs. This enables us to examine functional responses and vascular reactivity in presence of free and encapsulated drugs.



During 2012 we have significantly expanded our transgenic mice lines enabling the creation of inducible-global or cell specific knockouts (endothelial, pericytes and smooth muscle cells). These strains are available to support other programme lines in the analysis of gene function in vivo and the contribution of specific genes to disease pathogenesis. We have additionally developed semi-automated analysis protocols to quantify tissue remodelling using whole slide tissue slices stained by immunohistochemistry. We have continued our investigation of inflammatory processes underlying the pathogenesis of pulmonary hypertension.



Quantitative histology

Upper panels:
Vascular remodelling analysis, using dual von willebrand and smooth muscle actin staining

Lower panels:
Analysis of pulmonary fibrosis using masson's trichrome

Our future goal is to investigate our newly characterised liposomal formulations in an in vivo setting. To this end, we will profile the vasoreactivity of our new formulations in our isolated perfused lung systems to more accurately investigate pharmacokinetics. Data from these experiments will then be used to optimise drug release kinetics from liposomes.

Collaborations

Dr. Marsh is a participant and substitute member of the management committee for Austria for the COST Action: BM1201 Developmental Origins of Chronic Lung Disease
http://www.cost.eu/about_cost

GRUNIG Gabriele Ass.Prof., New York University School of Medicine, New York, US

HEINEMANN Akos Prof., Institute for Experimental and Clinical Pharmacology, Medical University Graz, Austria

KÖFELER Harald Dr., Medical University Graz, Austria

SCHULIGOI Rufina Prof., Institute for Experimental and Clinical Pharmacology, Medical University Graz, Austria

Publications 2012

Papers with LBI affiliation

Frascione D, Diwoky C, Almer G, Opriessnig P, Vonach C, Gradauer K, Leitinger G, Mangge H, Stollberger R, Prassl R. Ultrasmall superparamagnetic iron oxide (USPIO)-based liposomes as magnetic resonance imaging probes. Int J Nanomedicine. 2012, 7:2349-59. IF 3.130

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Kwapiszewska G, Markart P, Dahal BK, Kojonazarov B, Marsh LM, Schermuly RT, Taube C, Meinhardt A, Ghofrani HA, Steinhoff M, Seeger W, Preissner KT, Olschewski A, Weissmann N, Wygrecka M. PAR-2 inhibition reverses experimental pulmonary hypertension. Circ Res 2012, 110(9):1179-91. IF 9.489

Mosgoeller W, Prassl R, Zimmer A. Nanoparticle-mediated treatment of pulmonary arterial hypertension. Methods Enzymol. 2012, 508:325-54. IF 2.042

Veith C, Marsh LM, Wygrecka M, Rutschmann K, Seeger W, Weissmann N, Kwapiszewska G. Paxillin regulates pulmonary arterial smooth muscle cell function in pulmonary hypertension. Am J Pathol. 2012, 181(5):1621-33. IF 4.890

Papers without LBI affiliation

Hagner S, Welz H, Kicic A, Alrifai M, Marsh LM, Sutanto EN, Ling KM, Stick SM, Müller B, Weissmann N, Renz H. Suppression of adrenomedullin contributes to vascular leakage and altered epithelial repair during asthma. Allergy. 2012, 67(8):998-1006. IF 6.271

Books/ Book chapters

Prassl R. Lipoproteins „Role in Health and Diseases" Eds. Saša Frank, Gerhard Kostner.

Presentations at international conferences: posters

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Foris V, Kovacs G, Marsh L, Tscherner M, Olschewski A, Olschewski H. Phenotypical characterization of circulating endothelial progenitor cells in pulmonary hypertension.Österreichischen Gesellschaft für Pneumologie, June 2012, Salzburg, Austria.

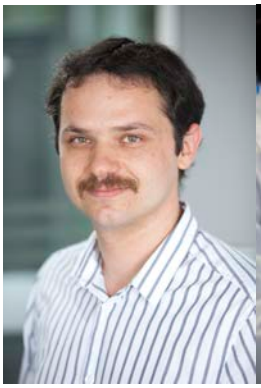
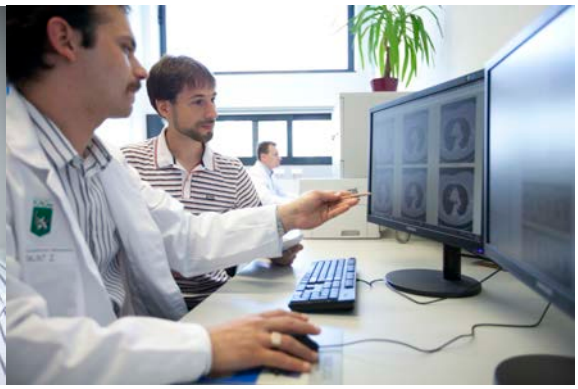
Marsh L, Zabini D, Xu H, Olschewski A, Kwapiszewska G. A potential role of HMGB1 in inflammatory vascular remodeling. 2nd Munich Lung Conference, Oct 2012, Munich, Germany.

Nagaraj C, Xu H, Olschewski A, Marsh L. Role of p22phox dependent NADPH oxidase in house dust mite (HDM) induce inflammatory response. UGMLC/DZL June 2012, Marburg, Germany.

Xu H, Zabini D, Olschewski A, Kwapiszewska G, Marsh LM. A potential role of high mobility group box-1 (HGMB1) in inflammatory vascular remodelling. UGMLC/DZL June 2012, Marburg, Germany.

2.3. Programme line C, Zoltán Bálint: Non-invasive diagnostics of pulmonary hypertension

Project Leader / Key Researcher
Name: Zoltán Bálint
Title: Dr.

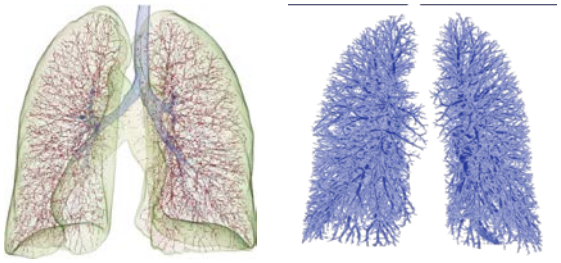



Group Members
Bálint Zoltán, Project leader
Fazakas Csilla, Ph.D. student
Helmberger Michael, Master student
Pienn Michael, Ph.D. student

Project Overview

This project line will provide a quantitative, non-invasive and reproducible technique with minimal user intervention for hemodynamic assessment of the pulmonary circulation by means of chest computed tomography (CT) imaging. The results will be compared with the results of standard examinations. Our primary objectives are to provide a reliable non-invasive assessment of regional pulmonary perfusion using chest CT in patients at risk for pulmonary hypertension (PH) and to provide assessment of cardiac output. Furthermore, we

want to distinguish pulmonary arterial hypertension (PAH) from pulmonary venous hypertension (PVH) as well as PAH from chronic thromboembolic PH (CTEPH).



These figures show lung tissue with vessels.

Research Results and Future Outlook

We have established the dual-energy CT data acquisition, protocol and image analysis. In 2012, a pilot study was performed and the acquired data were in good agreement with the expected values.

Cardiac output (CO) is an important diagnostic and prognostic factor in the hemodynamic evaluation of patients. The gold standard for CO measurement, thermodilution, requires an invasive right-heart catheterisation (RHC). The programme line has shown that CO determined with dynamic contrast-enhanced CT in the main pulmonary artery reliably predicts the values obtained by thermodilution during RHC. Moreover, from the same dynamic CT sequences the bolus propagation speed in the pulmonary artery correlates with the mean pulmonary artery pressure and this parameter can discriminate between patients with and without pulmonary hypertension. The easy determination of the propagation time and the comparably low dose for the examination makes this measure suitable for the routine clinical practice and can provide a basis for PH diagnosis. Therefore, this non-invasive technique might provide an alternative for repeated invasive right-heart catheter investigations in follow-up. This method was filed for patent application at the Austrian Patent Office on June 29th, 2012.

Pulmonary hypertension can result in a decrease of the blood vessel volume. The fractal dimension characterises the complexity of the lung vascular tree. Thus, this programme line developed an algorithm for automated lung vessel segmentation from contrast-enhanced CT images of the thorax. The plan for 2013 is to show that complexity readouts of the vessels are promising measures to determine certain hemodynamic parameters.

A software, which provides iodine quantification from dual energy CT images and was developed in 2012, will be tested in 2013. Here, the Siemens algorithm will be used as basis for comparison. For this purpose, phantom measurements with known concentrations of iodine were performed. A Matlab routine for impedance cardiography signal analysis was developed in cooperation with programme line D.

Steps for histogram analysis of the lung tissue, including the segmentation of the lung parenchyma and the search for optimal parameters, that describe changes between the different states of the disease, were made. In 2013, the development of the algorithm for lung segmentation and regional perfusion determination as well as the software development for volume histogram analysis will be continued. Finally, another project that will be initiated is the registration of the dynamic CT scans in order to determine local changes in the iodine concentration of the lung parenchyma.

Scientific Cooperations

JARAI-SZABO Ferenc Dr., Department of Physics, Babes-Bolyai University, Cluj-Napoca, Romania

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KULLNIG Peter Univ.-Doz. Dr., Diagnostikzentrum Graz für Computertomographie und Magnetresonanztomographie, Graz, Austria

NÉDA Zoltán Prof., Babes-Bolyai University, Cluj-Napoca, Romania

POPPER Helmuth Univ. Prof. Dr., Institute of Pathology, Medical University of Graz, Austria

SMOLLE-JÜTTNER Freyja-Maria Univ. Prof. Dr., Division of Thoracic and Hyperbaric Surgery, Department of Surgery, Medical University of Graz, Austria

STOLLBERGER Rudolf Prof., Technical University of Graz, Austria

URSCHLER Martin Dr., LBI for Clinic Forensic Imaging & Technical University of Graz, Austria

VARO György Dr., Institute of Biophysics, Biological Research Centre of the Hungarian Academy of Sciences, Szeged, Hungary

Publications 2012

Papers with LBI affiliation

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Fiorillo C, Moro F, Brisca G, Astrea G, Nesti C, Bálint Z, Olschewski A, Meschini MC, Guelly C, Auer-Grumbach M, Battini R, Pedemonte M, Romano A, Menchise V, Biancheri R, Santorelli FM, Bruno C. TRPV4 mutations in children with congenital distal spinal muscular atrophy. Neurogenetics 2012, 13(3):195-203. IF 3.488

Li Y, Connolly M, Nagaraj C, Tang B, Bálint Z, Popper H, Smolle-Juettner FM, Lindenmann J, Kwapiszewska G, Aaronson PI, Wohlkoenig C, Leithner K, Olschewski H, Olschewski A. Peroxisome proliferator-activated receptor-β/δ, the acute signaling factor in prostacyclin-induced pulmonary vasodilation. Am J Respir Cell Mol Biol. 2012, 46(3):372-9. IF 5.125

Presentations at international conferences: oral communications

Pienn M, Johnson TR, Kullnig P, Stollberger R, Olschewski A, Olschewski H, Bálint Z. Cardiac output determination by dynamic contrast-enhanced computed tomography. 20th Annual Meeting of the European Society of Thoracic Imaging, June 2012, London, UK.

Presentations at international conferences: posters

Pienn M, Bálint Z, Johnson TR, Kullnig P, Olschewski A, Olschewski H, Stollberger R. Simulation of cardiac output determination with dynamic contrast-enhanced computed tomography for definition of optimal settings. European Congress of Radiology, Mar 2012, Vienna, Austria.

Pienn M, Johnson TR, Kullnig P, Stollberger R, Olschewski A, Olschewski H, Bálint Z. Cardiac output determination by dynamic contrast-enhanced computed tomography. 20th Annual Meeting of the European Society of Thoracic Imaging, Jun 2012, London, UK.

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Pienn M, Johnson TR, Kullnig P, Stollberger R, Olschewski A, Olschewski H, Bálint Z. Cardiac output determination with dynamic contrast-enhanced computed tomography. 3rd Alpe-Adria Meeting on Pulmonary Hypertension, Oct 2012, Opatija, Croatia.

Patent

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2.4. Programme line D, Gabor Kovacs: Clinical data base and clinical studies

Project Leader / Key Researcher
Name: Gabor Kovacs
Title: Dr.

Group Members
Avian Alexander, Statistician
Foris Vasile, Ph.D. Student
Ghanim Bahil, Physician
Kovacs Gabor, Project leader
Nagler Lisa Maria, Study nurse
Olschewski Horst, Head of Department for Pulmonology
Tscherner Maria, Post Doc

Project Overview

The major task of this programme line is to manage an integrative clinical database of patients with pulmonary vascular diseases, meanly with pulmonary hypertension (PH) and at risk for the disease, including a biobank, where serum and plasma samples of well characterised patients are stored. We are also involved in the development and evaluation of new techniques for an early and non-invasive detection of PH in a broad population. A special focus of our group is the integration of physiological aspects in clinical science.

Research Results and Future Outlook

Integrative Database, Biobank, and Biomarkers

The establishment of an integrative database was one of the most important tasks. This provides easily accessible data from all patients, including complete diagnostic data at all important time points. Until now we have included approx. 900 patients in the database. Based on the data, retrospective generation of diagnostic and prognostic algorithms was performed and the prospective evaluation of such algorithms has been initiated. Main results were presented at international congresses in 2012. In 2013 and 2014 the further analysis of clinically relevant parameters and an algorithm development of non-invasive diagnostic and prognostic parameters are planned.

As part of the clinical database, serum, plasma and full blood of patients with PH and at risk for PH were reserved in the Biobank of the Medical University of Graz. The probes of the biobank are used in different studies of the programme lines A, B and D analysing the diagnostic and prognostic role of serum biomarkers in PH. A review of the current knowledge on the roles of biomarkers in PH written by Foris et al. was accepted by CHEST in 2012. The increase of atrial natriuretic peptide levels during exercise was associated with the increase of pulmonary arterial pressure in patients with connective tissue disease. These novel results were presented at international congresses last year. Several potentially clinically important serum biomarkers were analysed in 2012 in pilot studies. The results will be presented at the ATS Congress in Mai 2013. As a second step, larger validation studies are planned to be performed in 2013. In addition, further cooperation on the research of biomarkers is being prepared with other PAH centers within Austria.

The potential role of endothelial progenitor cells was investigated in patients with PH. The results were presented at international meetings in 2012. The further investigation of these cells is planned through establishment of an animal model in 2013.

Evaluation of innovative non-invasive methods

(Impedance Cardiography, Rebreathing, chest CT scan, MRI, ECG)

A commercial Impedance Cardiograph (CNSystems) is being tested in combination with invasive right heart catheterisation in PH patients. Investigations are performed during application of short-acting pulmonary vasodilators (if resting PAP >25mmHg). Until now, we have examined 25 patients. In cooperation with programme line C, the potential technical

errors of the method are addressed (mainly regarding the achievement of optimal signal and curves, signal averaging). Altogether, the inclusion of 76 patients is planned which should be achieved until June 2014.

Furthermore, we performed measurements using a commercially available inert gas rebreathing device (Innocor/Innovision, DK) in 26 patients. Our data showed a reasonable correlation of the data with invasively measured parameters, however they were less reliable in detecting minor changes during the pharmacologic testing. In addition, individual measurements showed large deviations compared to invasive measurements. Based on our experience and also the recommendation of the Scientific Advisory Board, we decided to stop the study at this stage. The results will be presented at the ATS Meeting in 2013.

Patients with PH and at risk for PH were investigated (including right heart catheterisation) and in cases in which the performance of a chest CT was clinically indicated, included into the chest CT evaluation trial of programme line C. Altogether, 25 patients were investigated. The results were presented at international congresses. In 2013, a second CT study is planned within the frames of a similar collaboration between us and programme line C.

The prospective evaluation of MRI based hemodynamic assessment of PH patients and its comparison with other non-invasive methods is planned in 2013. Before the initiation of the study, a cooperation with the Radiology of the MUG needs to be established.

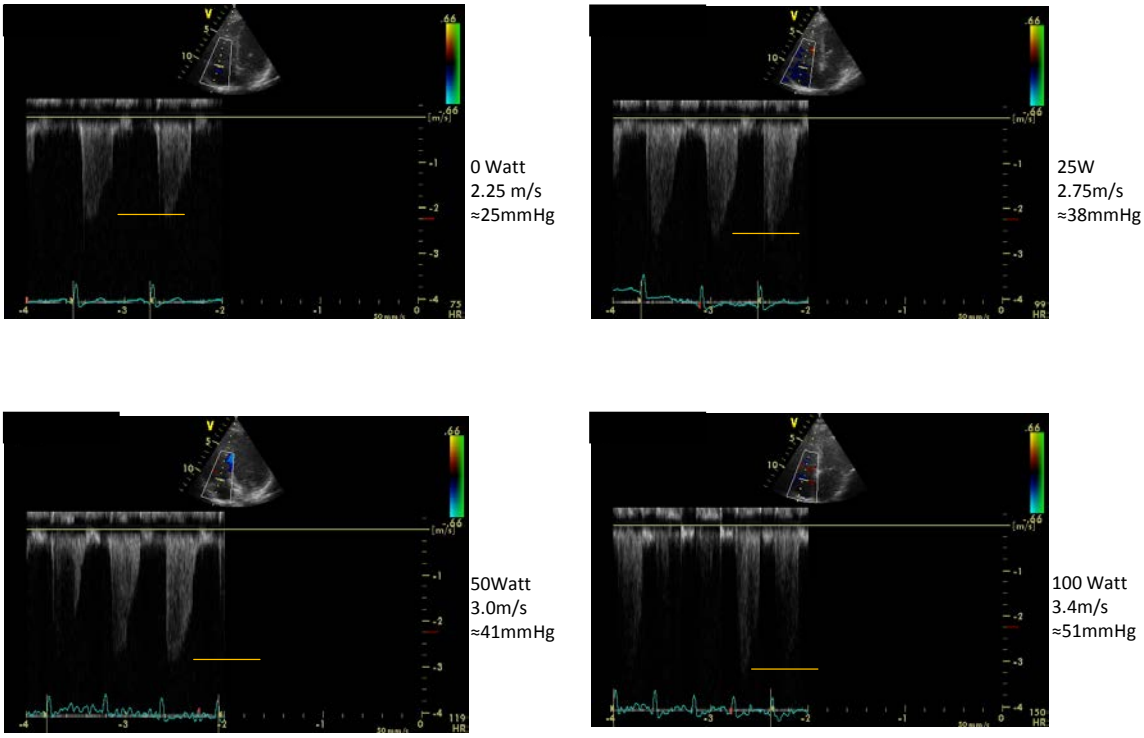
Based on a retrospective analysis, we determined a screening algorithm for PH using ECG and other simple non-invasive tools. The data of this retrospective study were presented at international congresses in 2012. At the time, the algorithm is being evaluated in a prospective manner. Results are expected by June 2013. These data may serve for the planning of a larger, population based study to define the role of ECG and other non-invasive tools in the diagnosis of PH.

Stress tests

A literature analysis has been performed by the LBI to describe the behaviour of pulmonary resistances during exercise in healthy subjects. This review may help to define normal ranges of resting and exercise pulmonary vascular resistance and was published in ERJ in 2012.

A follow up study has been performed in patients with connective tissue disease, who previously underwent exercise Doppler echocardiography or right heart catheterisation. We

included 65 patients with connective tissue disease 3-5 years after their initial scan. The study will be closed in May 2013.



Representative estimations of the systolic pulmonary arterial pressure during exercise-echocardiography performed in our scleroderma screening project

We aimed to create an algorithm that "corrects" 6-minute walk distance (6MWD) for the magnitude of subjective and objective exertion in order to improve the reliability of the test. A pilot study with 29 patients was performed and the results presented at international congresses in 2012. Initially, we planned the further multicenter evaluation of the test, however, based on the recommendation of the Scientific Advisory Board, we stopped the project at this stage. The submission of the manuscript containing the results of our pilot study is planned in the upcoming months.

Scientific Cooperations

ABERER Elisabeth Prof., Medical University of Graz, University Clinic for Dermatology and Venerology

BERGHOLD Andrea Prof., Alexander Avian, Medical University Graz, Institute for Medical Informatics, Statistics and Documentation

BRODMANN Marianne Prof., Medical University Graz, University Clinic for Internal Medicine, Division of Angiology

GRANINGER Winfried Prof., Medical University Graz, University Clinic for Internal Medicine, Division of Rheumatology

RAGGAM Reinhard Dr., Clinical Institute for Medical and Chemical Laboratory Diagnostics

SARGSYAN Karine Dr., Medical University Graz, Biobank

SILL Heinz Prof., Medical University Graz, University Clinic for Internal Medicine, Division of Hematology

STAUBER Rudolf Prof., Medical University Graz, University Clinic for Internal Medicine, Division of Gastroenterology

Publications 2012

Papers with LBI affiliation

Kovacs G, Olschewski A, Berghold A, Olschewski H. Pulmonary vascular resistances during exercise in normal subjects: a systematic review. Eur Respir J. 2012, 39(2): 319-28. IF 5.895

Kovacs G, Connolly M. Pulmonary hypertension: a guide for GPs. Br J Gen Pract. 2012, 62(604):e795-7. IF 1.831

Kovacs G, Olschewski H. Pulmonale Hypertonie. Dtsch Med Wochenschr 2012, 137: 2026-28. IF 0.528

Kovacs G, Olschewski H. Pulmonale Hypertonie bei häufigen Lungenkrankheiten. Klinik Orphan Diseases 2012, 37-38. – no IF, not peer-reviewed

Kovacs G, Flick H, Fürst F, Olschewski H. Rheumatologie und Pneumologie. Was der Pulmologe wissen muss. CliniCum Pneumo 2012, 5(12):6-12. -- no IF, not peer-reviewed

Papers without LBI affiliation

Kovacs G, Maier R, Aberer E, Brodmann M, Graninger W, Kqiku X, Scheidl S, Tröster N, Hesse C, Rubin L, Olschewski H. Pulmonary arterial hypertension therapy may be safe and effective in patients with systemic sclerosis and borderline pulmonary artery pressure. Arthritis Rheum. 2012, 64(4):1257-62.

Presentations at international conferences: posters

Tscherner M, Kovacs G, Fruhwald F, Maier R, Obermayer-Pietsch B, Foris V, Olschewski H. The proANP increase during exercise may predict the PAP increase in connective tissue

disease patients at risk of PAH. Jahreskongress der Österreichischen Gesellschaft für Kardiologie, May 2012, Salzburg, Austria.

Foris V, Kovacs G, Marsh L, Tscherner M, Olschewski A, Olschewski H. Phenotypical characterization of circulating endothelial progenitor cells in pulmonary hypertension. Jahrestagung der Österreichischen Gesellschaft für Pneumologie, June 2012, Salzburg, Austria.

Kovacs G, Foris V, Tscherner M, Avian A, Kqiku X, Olschewski A, Olschewski H. Routine non-invasive parameters in the practical diagnostic work-up of patients with risk for pulmonary hypertension. Jahrestagung der Österreichischen Gesellschaft für Pneumologie, June 2012, Salzburg, Austria.

Kovacs G, Stöckl C, Avian A, Kqiku X, Foris V, Tscherner M, Olschewski H. The interpretation and clinical relevance of heart rate increase during the six-minute walk test in pulmonary hypertension. Jahrestagung der Österreichischen Gesellschaft für Pneumologie, June 2012, Salzburg, Austria.

Tscherner M, Kovacs G, Foris V, Scheidl S, Avian A, Olschewski A, Olschewski H. End-tidal CO2 pressure may facilitate differential diagnostics between PH patients with chronic heart or lung disease and CTEPH. Jahrestagung der Österreichischen Gesellschaft für Pneumologie, June 2012, Salzburg, Austria.

Tscherner M, Kovacs G, Fruhwald F, Maier R, Obermayer-Pietsch B, Foris V, Olschewski H. The proANP increase during exercise may predict the PAP increase in connective tissue disease patients at risk of PAH. Jahrestagung der Österreichischen Gesellschaft für Pneumologie, June 2012, Salzburg, Austria.

Foris V, Kovacs G, Marsh L, Tscherner M, Olschewski A, Olschewski H. Fluorescence activated cell sorting for simultaneous assessment of nine surface markers of endothelial progenitor cells in pulmonary hypertension.European Respiratory Society Annual Congress, Sept 2012, Vienna, Austria.

Kovacs G, Foris V, Tscherner M, Avian A, Kqiku X, Olschewski A, Olschewski H. The clinical role of routine non-invasive parameters in the diagnostic work-up of patients with risk for pulmonary hypertension. European Respiratory Society Annual Congress, Sept 2012, Vienna, Austria.

Kovacs G, Stöckl C, Avian A, Kqiku X, Foris V, Tscherner M, Olschewski H. The clinical relevance of heart rate increase in the interpretation of six-minute walk test in pulmonary hypertension. European Respiratory Society Annual Congress, Sept 2012, Vienna, Austria.

Tscherner M, Kovacs G, Foris V, Scheidl S, Avian A, Olschewski A, Olschewski H. End-tidal CO2 pressure may facilitate differential diagnostics between PH patients with chronic heart or lung disease and CTEPH. European Respiratory Society Annual Congress, Sept 2012, Vienna, Austria.

Tscherner M, Kovacs G, Fruhwald F, Maier R, Obermayer-Pietsch B, Foris V, Olschewski H. The proANP increase during exercise may predict the PAP increase in connective tissue disease patients at risk of PAH. European Respiratory Society Annual Congress, Sept 2012, Vienna, Austria.

Invited talks

Kovacs G. Pulmonale Zirkulation. ÖGP Summer School, June 2012, St. Gilgen, Austria.
Kovacs G. PVR und TPR in Ruhe und bei Belastung bei Gesunden. DACH Symposium, Oct 2012, Heidelberg, Germany.
Kovacs G. Pulmonary exercise hemodynamics of the healthy. Sport and Congenital Heart Disease Congress, Nov 2012, Munich, Germany.

Awards

Foris V. Short Term Fellowship. Österreichische Gesellschaft für Pneumologie, June 2012, Salzburg, Austria.
Kovacs G. Pfizer Young Researcher Award 2012.
Tscherner M. 3rd Poster Award. Österreichische Gesellschaft für Pneumologie, June 2012, Salzburg, Austria.

3. Other Activities

3.1. Teaching activities

Since 2008 **Dr. Andrea Olschewski** has been the Dean for Doctoral Studies at the Medical University of Graz. She is responsible for the development of the PhD programmes and has led and successfully finalised the international accreditation of the PhD study of the Medical University of Graz in 2011. Andrea Olschewski is involved in teaching at different levels at the Medical university of Graz. She gives student lectures in the Human Medicine Diploma Program (Anaesthesiology, Intensive Care Medicine and Pain therapy) and seminars for doctoral studies. She supervises either as a principal investigator or the member of the dissertation comittee of all PhD students of the LBI for Lung Vascular Research. Furthermore, Andrea Olschewski has successfully finished the supervision of two PhD students, Yingji Li and Patrick Chorlich, at the Justus-Liebig University of Giessen, Germany.

Dr. Grazyna Kwapiszewska taught between 2005 and 2010 within the frame of Graduate School “Molecular Biology and Medicine of the Lung“ at the University Giessen Lung Center. She supervised two PhD students at the University Giessen Lung Center, Christine Veith and Friderike Weisel, who both successfully did their promotion exams in 2012. Currently, Dr. Grazyna Kwapiszewska supervises one PhD Student at the LBI-LVR in Graz, Miss Valentina Biasin.

Dr. Leigh Marsh is a member of the thesis committee for Pritesh Jain, Hui Xu and Lazlo Foris. Ruth Prassl is the supervisor of Bernhard Lehofer.

Dr. Zoltán Bálint is currently co-supervising PhD student Michael Pienn and MSc student Michael Helmberger.

Dr. Gabor Kovacs is currently supervising PhD student Vasile Foris. In addition, he gives student lectures in the Human Medicine Diploma Program (Pulmonology) and supervises Adamowitz Sebastian who writes his diplom work on the relevance of DlCO measurements in different pulmonary diseases including PH.

3.2. Internal activities of the Institute

Within the LBI-LVR institute, several seminar series and regular lab meetings are organised to improve group communication and scientific knowledge:

Key Researcher Meetings

The LBI-LVR key researchers meet once in a month to discuss about budget and task distribution, research and various other open issues for the benefit of the institute. For internal organisation, a protocol is made in written form accessible to the key researchers.

Weekly Seminars

The LBI-LVR members and associated researchers meet once in a week at a seminar. There one or two researchers display their projects, including discussion and troubleshooting, to the whole team.

Lab Meetings

Every programme line leader has a weekly meeting with the group members in order to discuss project development, publication strategy, and group organisation.

Journal Club Seminars

All four programme lines of the institute have a weekly journal club seminar. There a topic related paper is presented and discussed. The seminars are implemented into the PhD Programme Molecular Medicine of the MUG and so also several other PhD students join them.

Institute Meetings

The LBI-LVR and associate members meet four times per year in order to discuss organisational issues of the institute, task distribution, research and various other topics.

Group member meetings

The LBI-LVR members meet every month in order to discuss issues of the organisation of the institute, task distribution, research and various other open issues. The frequency of these meeting are adapted to the needs of the institute.

Invited Speakers

Name	Affiliation	Date	Title of the Talk
WITZENRATH Martin Prof.	Department of Infectious Diseases and Respiratory Medicine at Charité – Universitätsmedizin Berlin, Germany	Jan 2012	Th2 inflammation in mice: a suitable model for PAH?
GRUNIG Gabriele Ass.Prof.	New York University School of Medicine, New York, US	Feb 2012	Inflammatory response in pulmonary arterial remodelling
CZIRJÁK László Prof.	Department of Immunology and Rheumatology of the University of Pécs, Hungary	June 2012	Epidemiology and different manifestation of collagenoses
KOMÓCSI András Dr.	Department of Immunology and Rheumatology of the University of Pécs, Hungary	June 2012	Cardiac and pulmonary-vascular participation of scleroderma
FALUDI Réka Dr.	Department of Immunology and Rheumatology of the University of Pécs, Pécs, Hungary	June 2012	Diastolic dysfunction in scleroderma patients
NÉDA Zoltán Prof.	Department of Physics, Babes-Bolyai University, Cluj-Napoca, Romania	June 2012	The unexpected rhythm
WEISSMANN Norbert Prof.	ECCPS, Giessen, Germany	June 2012	Inhibition of iNOS reverses tobacco smoke-induced lung emphysema and pulmonary hypertension in mice
WILHELM Jochen Dr.	University Giessen Lung Center, Giessen, Germany	July 2012	Statistical course
THORSTEN Johnson PD Dr.	Department of Diagnostic Radiology, Ludwig-Maximilians-University, Munich, Germany	Oct 2012	Dual-energy computed tomography

Team building activities in the dripstone show cave Katerloch

In the end of June, all members of the LBI-LVR participated in a team building activity. We went to the dripstone show cave Katerloch close to Dürntal/ Weiz. It was amazing to see a world full of fantastic rock formations, stalagmites and stalactites. This event was a nice opportunity to get together and improve the team spirit by discovering the cave and enjoying the typical Austrian food in a tavern in Dürntal. After refreshment, the participants were hiking together a distance in the Weizklamm.