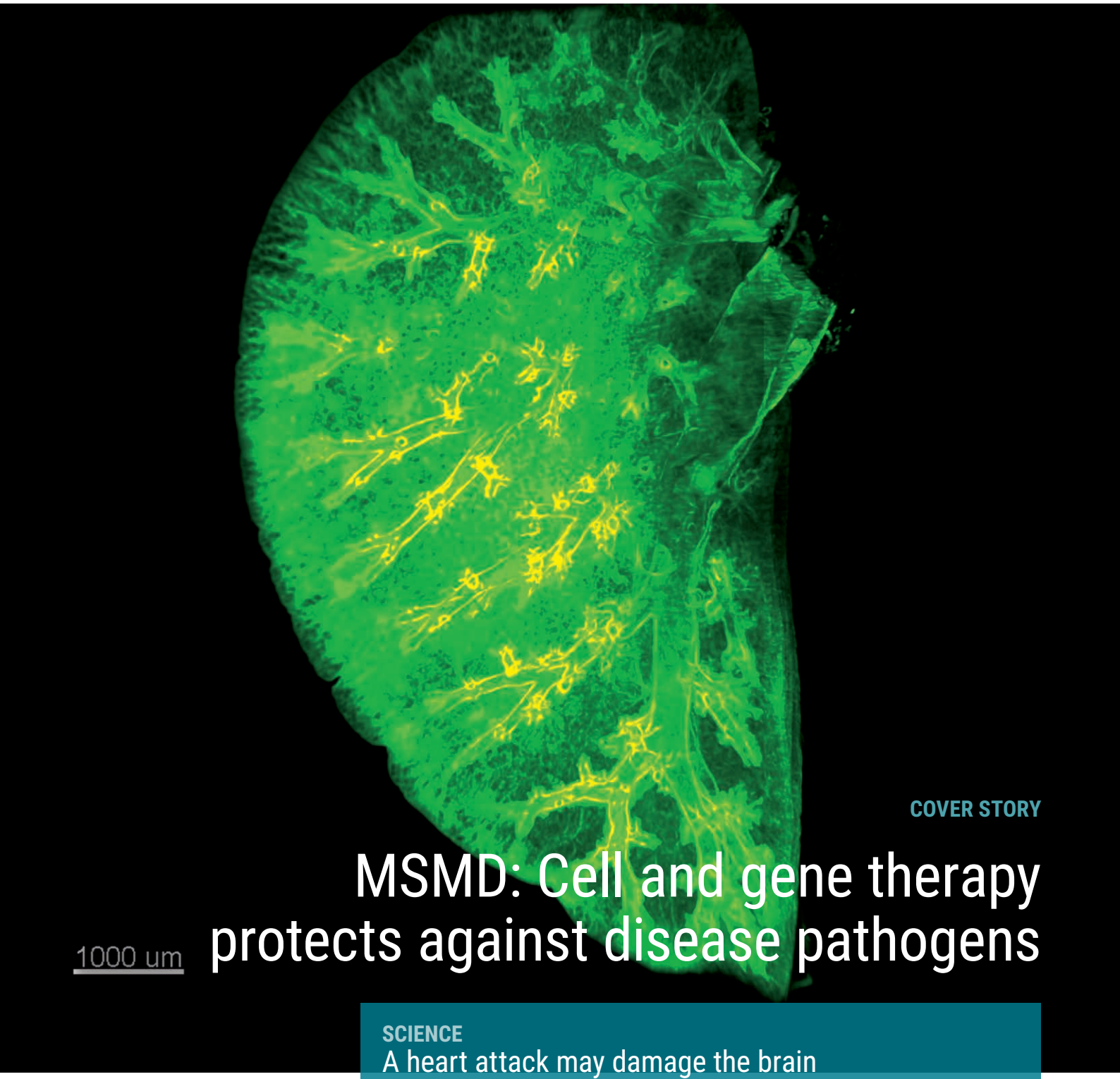
rebirth news 1 JULY 2018



COVER STORY

MSMD: Cell and gene therapy protects against disease pathogens

Latest Publications

MicroRNAs have therapeutic and prognostic value in leukaemia

The German Cancer Consortium (DKTK) has, with the involvement of REBIRTH, found a ribonucleic acid (RNA) that indicates the success of leukaemia treatment and may also act as a therapeutic agent. In addition to Hannover Medical School (MHH), the consortium also includes both Frankfurt and Ulm University Hospitals. Small RNA molecules called miRNA-193b order the cancer cells to self-destruct and make them more sensitive to drug action.

Publication

Bhayadia R, Krowiorz K, Haetscher N, Jammal R, Emmrich S, Obulkasim A, Fiedler J, Schwarzer A, Rouhi A, Heuser M, Wingert S, Bothur S, Döhner K, Mätzig T, Ng M, Reinhardt D, Döhner H, Zwaan CM, Eibrink MV, Heckl D, Fornerod M, Thum T, Humphries RK, Rieger MA, Kuchenbauer F, Klusmann JH. Endogenous Tumor Suppressor Microrna-193b: Therapeutic and Prognostic Value in Acute Myeloid Leukemia. Journal of Clinical Oncology. 2018;36(10):1007.

Platform for exploring new therapeutic approaches to MSMD

An interdisciplinary REBIRTH team has developed a new disease model for a rare blood condition called Mendelian susceptibility to mycobacterial disease (MSMD), in which the scavenger cells (macrophages) can no longer eliminate pathogens that have entered the body. They took cells obtained from various patient samples and reprogrammed them into induced pluripotent stem cells (iPSCs). The macrophages generated from the iPSCs provided insights into how the disease forms and have potential for developing new treatments.

Publication

Neehus AL*, Lam J*, Haake K, Merkert S, Schmidt N, Mucci A, Ackermann M, Schubert M, Happle C, Kuhnel MP, Blank P, Philipp F, Goethe R, Jonigk D, Martin U, Kalinke U, Baumann U, Schambach A, Roesler J, Lachmann N (*equal contribution. Impaired IFNγ-Signaling and Mycobacterial Clearance in IFNγ1-Deficient Human iPSC-Derived Macrophages. Stem Cell Rep. 2018;10:7-16.

What effect does laser nanosurgery have on cells?

To enhance understanding of how femtosecond laser nanosurgery affects cells, REBIRTH researchers used this technique to manipulate skeletal muscle cells. They made cuts of different lengths and compared stained and unstained cells. The scientists analysed how the cuts healed and the effect on survival rate and morphology. They discovered that, to avoid unwanted cell responses, the laser parameters must be chosen carefully, depending on the staining of the cell, its (differentiation) state, and the extent of the cut region.

Publication

Hagenah D, Heisterkamp A, Kalies S. Effects of Cell State and Staining on Femtosecond Laser Nanosurgery. J Biophotonics. 2018;10.1002/jbio.201700344:e201700344. Epub 2018/02/21.

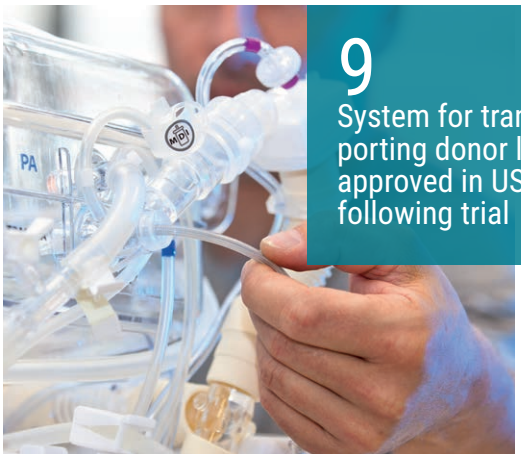
New method for predicting maturation of T cells

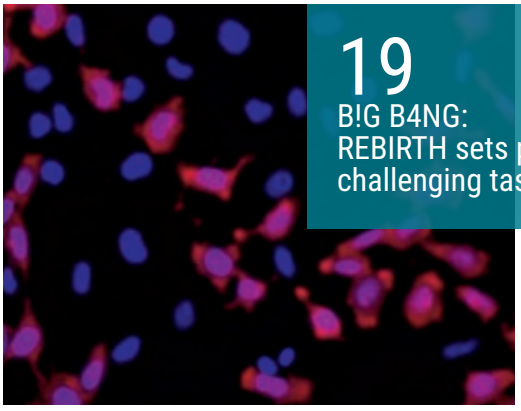
Mice transplanted with human cord blood-derived hematopoietic stem cells (HSCs) can be used by researchers to characterize the steps in reconstitution of the human immune system. In this study, REBIRTH researchers investigated whether humanized female and male mice exhibit different patterns of T cell response. They developed a set of multidimensional analyses with which they were able to characterize the immunization status of mice in lymphatic tissue. Working with bioinformatics professionals, they trained an artificial neural network with immunophenotypic markers to predict how humanized mice respond to immunization.

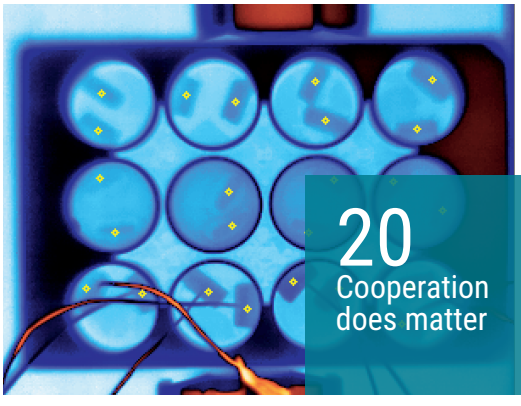
Publication

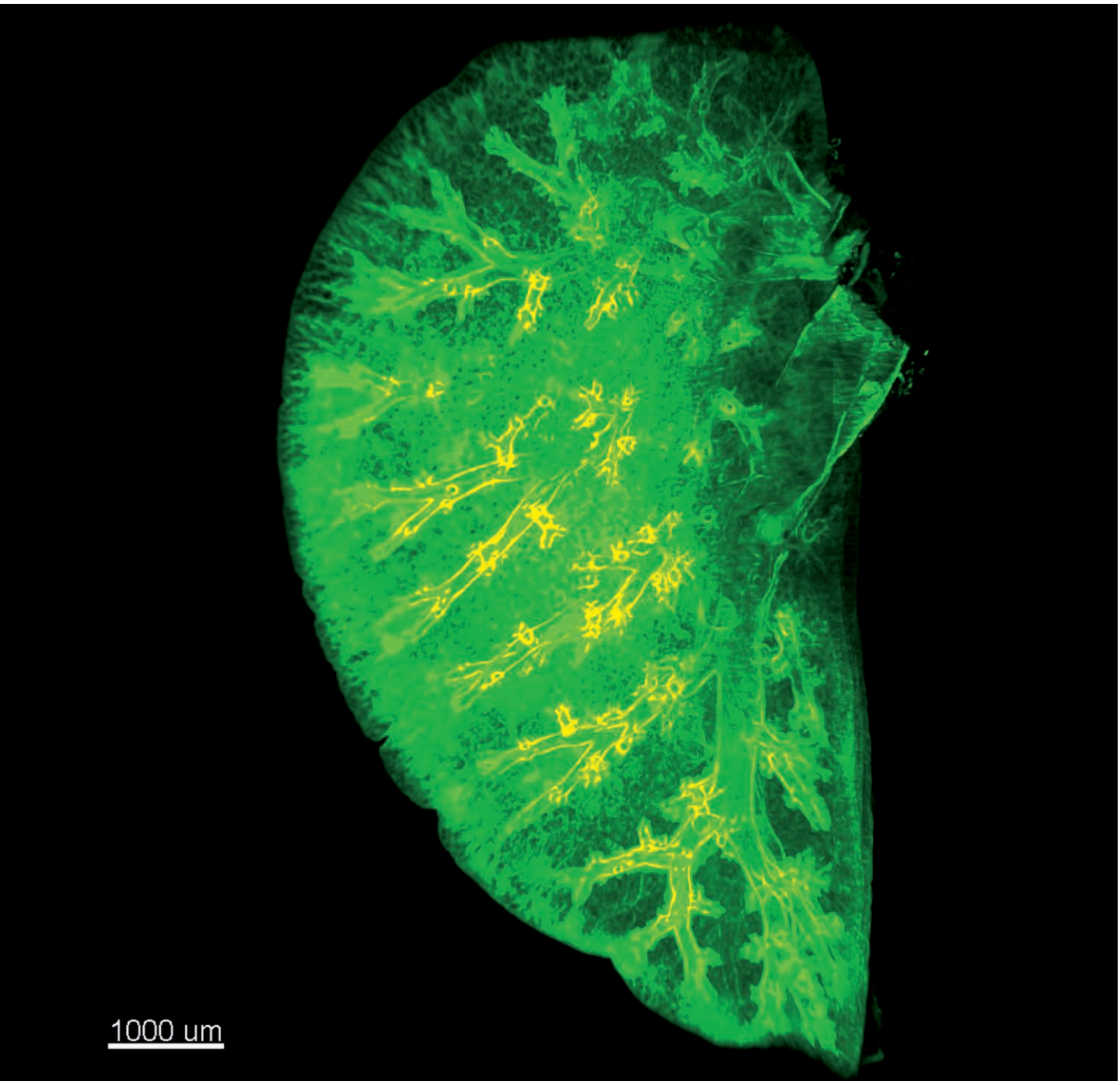
Volk V., Reppas A.I., Robert P.A., Spineli L.M., Sundarasetty B.S., Theobald S.J., Schneider A., Gerasch L., Deves Roth C., Kloss S., Koehl U., von Kaisenberg C., Figueiredo C., Hatzikirou H., Meyer-Hermann M., Stripecke R. Multidimensional Analysis Integrating Human T-Cell Signatures in Lymphatic Tissues with Sex of Humanized Mice for Prediction of Responses after Dendritic Cell Immunization. Front Immunol 2017; 8:1709.

Contents









This image shows the structure (green) of part of the lung of a mouse treated with genetically corrected stem cells. No mycobacterial infiltration is evident.

MSMD: Cell and gene therapy protects against disease pathogens

The human immune system is equipped with a wide variety of blood cells that enable it to combat different pathogens on a daily basis. Weakening of the immune system can lead to patients suffering from serious infectious diseases. One cause of this may be DNA mutations that lead to the body's scavenger cells (macrophages) no longer working, which means that pathogens such as mycobacteria can no longer be properly eliminated. One situation in which this occurs is where MSMD is present; here, patients are affected by severe infections (which, in some cases, prove fatal) caused by mycobacteria. 'Mendelian susceptibility to mycobacterial disease' (MSMD) is a genetic condition that manifests itself in early childhood, and for which no effective curative treatment is yet available.

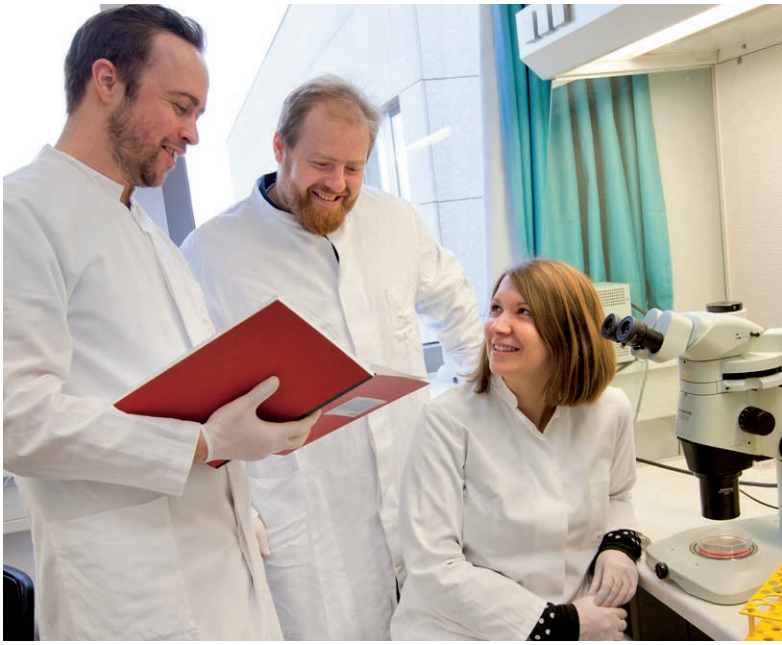
An international team led by Dr Nico Lachmann (from the Institute of Experimental Haematology and the REBIRTH Unit Translational Haematology of Congenital Diseases) has now, and for the first time, paved the way for an effective and sustained therapy for MSMD. "For the most serious form of MSMD, namely IFNgR1 deficiency, we have developed gene vectors that allow us to correct the genetic defect in the target cells and thus restore macrophage function," says Dr Lachmann. The team of scientists and physicians from Hannover, Italy, France and the USA has already succeeded in demonstrating the efficacy of this novel therapy in a mouse disease model. "The treatment works so well that only a few genetically corrected cells are needed to protect the entire organism from serious mycobacterial infections," adds Dr Miriam Hetzel, alumna of the PhD programm Regenerative Sciences (RegSci) who shares lead authorship with Dr Adele Mucci (formerly of RegSci/MHH, now at the San Raffaele Hospital in Milan) and Patrick Blank of TWINCORE. After being transplanted, the genetically corrected blood stem cells migrate into the bone marrow where they immediately begin to form the scavenger cells that have been lacking. The investigators were surprised that the newly formed scavenger cells subsequently migrated very speedily into the various organs to do their job there. "We were delighted at how effectively and rapidly this therapy exerts its protective effect," comments Dr Lachmann. The fact that only a small number of corrected cells are needed for this treatment gives cause for hope that it can soon be transferred into clinical practice. "Building on these findings, we are currently working on replacing only the defective scavenger cells with healthy cells in the lungs of MSMD mice. This form of cell-based therapy will then open up whole new horizons in regenerative medicine," Dr Lachmann says.

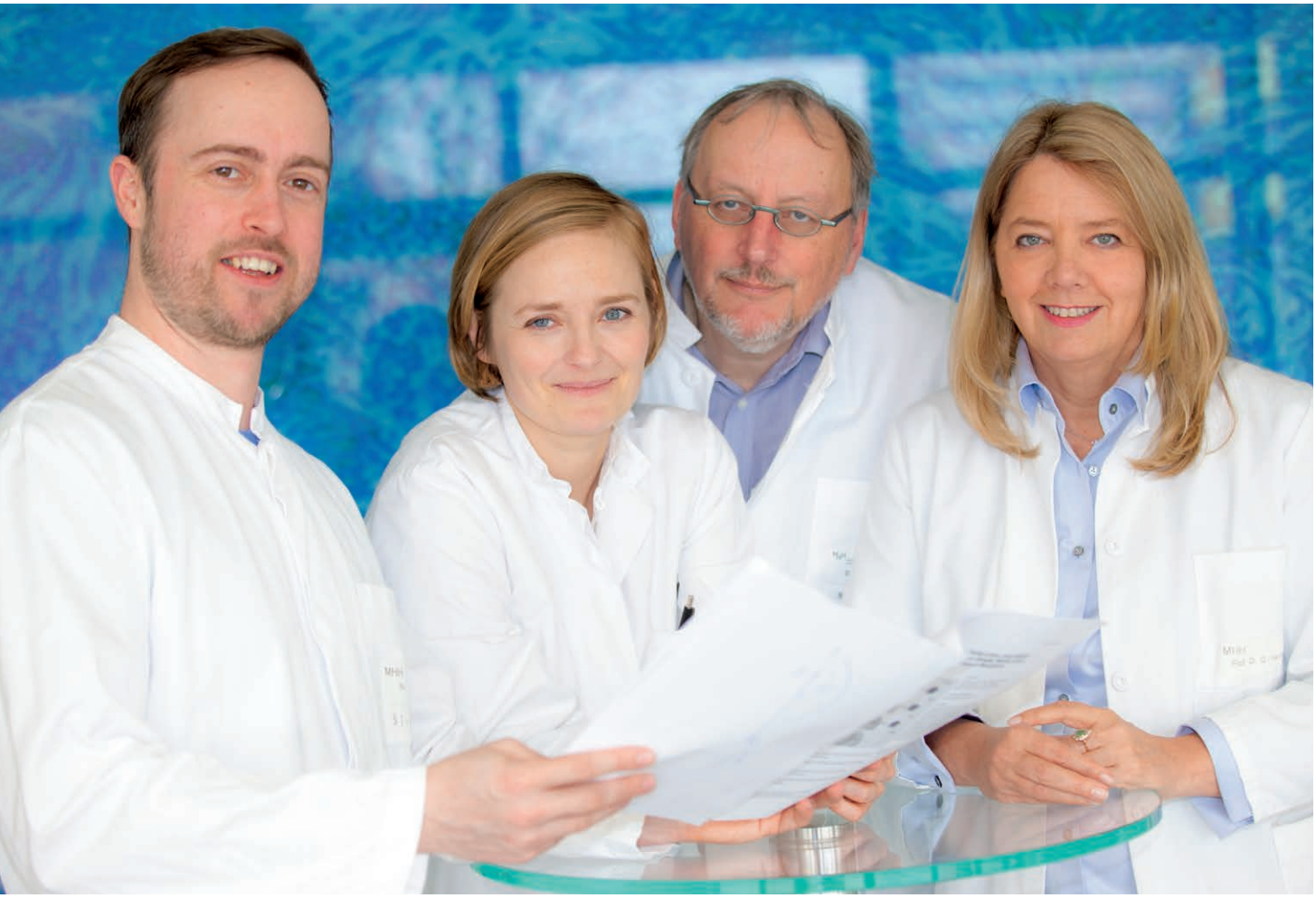
Publication

Hetzel M, Mucci A*, Blank P*, Nguyen AHH, Schiller J, Halle O, Kuhnel MP, Billig S, Meineke R, Brand D, Herder V, Baumgartner W, Bange FC, Goethe R, Jonigk D, Forster R, Gentner B, Casanova JL, Bustamante J, Schambach A, Kalinke U, Lachmann N. Hematopoietic Stem Cell Gene Therapy for Ifngammar1 Deficiency Protects Mice from Mycobacterial Infections. Blood. 2018;131(5):533-45. Epub 2017/12/14. (*co-authorship)*

> *"This form of cell-based therapy will then open up whole new horizons in regenerative medicine."*

Dr Nico Lachmann with two of the three lead co-authors: Patrick Blank and Dr Miriam Hetzel.





Dr Nico Lachmann (REBIRTH unit on Translational Haematology of Congenital Diseases), Dr Christine Happle, Professor Thomas Moritz (REBIRTH unit on iPS-based haematopoietic Regeneration) and Professor Gesine Hansen (from left).

An exciting prospect: patients cured by their own cells?

Altering patients' own cells so they can replace defective or absent cells – researchers at Hannover Medical School (MHH) have moved a step closer to achieving this. The scientists are investigating hereditary pulmonary alveolar proteinosis (herPAP), a rare, life-threatening condition in which the scavenger cells (macrophages) in the lungs are defective. To replace them, the researchers produced what are called induced pluripotent stem cells (iPS cells) from mature human cells. These cells were matured into scavenger cells in the laboratory and then transplanted into the lungs of diseased mice. These animals' immune system had been modified so that it resembles that of humans and helped the cells become implanted in the lung. And this proved a resounding success: the cells adapted to the special environment of the lung, the condition improved, and no significant side effects occurred.

"Our aim is that, in the future, our new therapeutic approach can contribute to a cure for these seriously ill children," says Professor Gesine Hansen, director of the Department of Paediatric Pulmonology, Allergology and Neonatology. The intention: to make it possible to create patient-specific macrophages developed from iPS cells. These could, following gene correction *in vitro*, mature into scavenger cells and subsequently be transplanted directly into the lung. A clinical study is currently being planned in America in which this approach is to be transferred to humans by using blood stem cells. The research team in Hannover is to be the European partner in this investigation. The scientists from the German Center for Lung Research (DZL) and the REBIRTH Cluster of Excellence assume that the findings will be applicable to other diseases. The new method would be less risky for patients than transplanting either gene-corrected stem cells or bone marrow

What is pulmonary alveolar proteinosis?

Hereditary pulmonary alveolar proteinosis (herPAP) is a rare, life-threatening lung disease. To date, fewer than 100 cases have been described worldwide; in Germany, only a handful of children are affected. Protein-rich material accumulates in the tiny sacs (alveoli) in the lungs, which normally contain air. This is usually removed by scavenger cells (macrophages), but these do not work properly in PAP sufferers. Many of those affected die from asphyxiation in childhood. As yet there is no treatment that combats the causes of this disease. The only current therapy option is pulmonary lavage, which must be performed every four weeks or so under general anaesthetic. Treatment lasts a long time and is risky.

Publication

Happle C, Lachmann N, Ackermann M, Mirenska A, Göhring G, Thomay K, Mucci A, Hetzel M, Glomb T, Suzuki T, Chalk C, Glage S, Dittrich-Breiholz O, Trapnell B, Moritz T, Hansen G. Pulmonary Transplantation of Human Ipsc-Derived Macrophages Ameliorates Pulmonary Alveolar Proteinosis. Am J Respir Crit Care Med. 2018;10.1164/rccm.201708-1562OC. Epub 2018/04/14.

Organ Care System: physicians treat diseased lung outside the body

Successfully attempted in animals: therapeutic option for severe pneumonia with 100-fold antibiotic dosage

The growing resistance of pathogens to antibiotics means that, in an increasing number of cases, doctors are out of alternative treatment options for severe pneumonia. In this pilot study for the first time, Medics at Hannover Medical School (MHH) and Veterinarians at the University of Veterinary Medicine Hannover, Foundation, have used the Organ Care System for successful treatment of pneumonia caused by drug-resistant bacteria outside the body. The advantage: when the lung is removed for treatment purposes, physicians can use very high doses of antibiotics that would be toxic to the body, with severe side effects.

The investigators treated affected animals with a systemically tolerable dose of the antibiotic colistin and, using the OCS, also treated the explanted diseased lower left lung (LLL) with colistin at 100 times the strength. "Such a dose would not be tolerated by the patient, leading to kidney failure and damage to the central nervous system," explains Dr Norman Zinne, a specialist at MHH-Department of Cardiothoracic, Transplantation and Vascular Surgery (HTTG). Colistin has been seeing increasing use as a reserve antibiotic for treatment of pneumonia.

To treat the lung infection outside the body, the doctors first explanted the affected part of the lung and implanted it into an OCS, which is a mobile device for perfusing the lung *ex vivo*. This system enables donor organs to be transported at body temperature, perfused by donated blood and supplied with nutrients – or, as here, antibiotics. The lung is ventilated during treatment, enabling it to supply itself with oxygen.

Dr Norman Zinne and Professor Axel Haverich with an Organ Care System (OCS) containing a pig's lung; Professor Haverich is holding the control unit for the OCS.

After the two-hour procedure, the medics reimplanted the LLL in the animal. "Whereas only one-third of the pigs in the untreated control group and the conventionally treated group survived their pneumonia, two-thirds of the animals whose lungs we treated extracorporeally recovered from the disease," Dr Zinne says. Furthermore, the clinical symptoms of the infection were less serious in the OCS group than in the other groups. "The results show that treatment of multi-resistant pneumonia using very high-dose antibiotics outside the body constitutes a new therapeutic strategy for severe infections where alternative treatments are now lacking," adds Professor Haverich, director of HTTG. "Before we can apply this method to people we will, for patient safety reasons, first carry out further studies using an animal model," stresses the REBIRTH coordinator. "It is quite conceivable that this approach can be transferred to different organs such as the heart, or to other treatment options such as chemotherapy or cell therapies."

The project was carried out in close collaboration with the Professor Waldmann's and Dr. Doris Höltig's Team at Clinic for Swine, Small Ruminants and Forensic Medicine at University of Veterinary Medicine Hannover, Foundation.

Publication

Zinne N., Krueger M., Hoeltig D., Tuemmler B., Boyle E.C., Biancosino C., Hoeffler K., Braubach P., Rajab T.K., Ciubotaru A., Rohde J., Waldmann K.H., Haverich A. Treatment of infected lungs by ex vivo perfusion with high dose antibiotics and autotransplantation: A pilot study in pigs. PLoS One 2018; 13:e0193168.

Close-up of an OCS.

System for transporting donor lungs approved in USA following trial

In a clinical trial called INSPIRE, a large international team of investigators led by physicians at Hannover Medical School (MHH) has shown that the transport and storage of donor lungs using the Organ Care System (OCS) – developed by Transmedics, Inc. – is safer and more effective than the current standard procedure, namely storage at four degrees Celsius. Another clinical benefit: patients who received an organ preserved using the OCS had a lower incidence of primary graft dysfunction (a common complication with cold storage, and which usually occurs within the first 72 hours). "The potential implications for patients are shorter ventilation periods and more rapid discharge," says Professor Gregor Warnecke, chief physician (*leitender Oberarzt*) at MHH's Department of Cardiothoracic, Transplantation and Vascular Surgery (HTTG) and the study's principal investigator. Twenty-one transplant centres were involved in this international trial, in which MHH treated the largest patient group.

In late March, based on the study's outcome, the United States Food and Drug Administration (USFDA) granted approval for the OCS for storage of lungs for transplantation.

Advantages of the Organ Care System

The OCS is a mobile device for perfusing the lung *ex vivo*. This system enables donor organs to be transported at body temperature, perfused by a blood-like solution and supplied with nutrients. The lung is ventilated in the OCS, enabling it to supply itself with oxygen. The device gives clinicians up to 12 hours in which to transport the donor organ, and to assess and improve pulmonary function – mucous and retained fluid can be removed, for instance. "This means the organ reaches the recipient in considerably better condition than when (as previously) kept in cold storage," says Professor Warnecke. "And surgeries can be planned more effectively, with ultimate benefits in terms of patient safety." HTTG director Professor Axel Haverich adds: "The number of organs donated in Germany is continuously decreasing. The OCS allows us to implant donor organs here in Hannover from further afield in Europe than was previously possible."

The previous standard method of transport, at four degrees Celsius, gives the transplant physicians only a 10-hour window, during which the lack of a blood and nutrient supply means the organ continues to deteriorate. The result, in up to 30% of patients, is post-transplant primary graft dysfunction: lung function worsens, and this may prove fatal.

Various aspects of the research and further development of *ex vivo* lung perfusion were supported by the three major transplantation research alliances at MHH: the REBIRTH Cluster of Excellence, the German Center for Lung Research (DZL), and the Integrated Research and Treatment Centre Transplantation (IFB-Tx).

Publication

Warnecke G., Van Raemdonck D., Smith M.A., Massard G., Kukreja J., Rea F., Loor G., De Robertis F., Nagendran J., Dhital K.K., Moradiellos Diez F.J., Knosalla C., Bermudez C.A., Tsui S., McCurry K., Wang I.W., Deuse T., Leseche G., Thomas P., Tudorache I., Kuhn C., Avsar M., Wiegmann B., Sommer W., Neyrinck A., Schiavon M., Calabrese F., Santelmo N., Olland A., Falcoz P.E., Simon A.R., Varela A., Madsen J.C., Hertz M., Haverich A., Ardehali A. Normothermic ex-vivo preservation with the portable Organ Care System Lung device for bilateral lung transplantation (INSPIRE): a randomised, open-label, non-inferiority, phase 3 study. Lancet Respir Med 2018; 6:357.

A heart attack may damage the brain

Even a heart attack without complications can adversely affect the brain. This was the conclusion drawn by scientists at Hannover Medical School (MHH). Using state-of-the-art imaging techniques, the team – headed by Professor Frank Bengel, director of MHH's Department of Nuclear Medicine and REBIRTH unit on Radionuclide Molecular Imagine – demonstrated that a myocardial infarction (heart attack) precipitates not only inflammation of the cardiac muscle but also an inflammatory response in the brain (neuroinflammation).

The inflammation of the heart muscle that takes place following a heart attack is supposed to aid the healing process. If the reaction is excessive, however, it leads to further damage to, and deterioration in, cardiac function (i.e. heart failure). It was previously assumed that this process is chiefly localized to the heart itself. Findings by the MHH investigators now show, however, that immediately following an infarction – and in cases where heart failure subsequently develops – the brain is also affected. This interconnectedness between heart and brain is thought to be mediated by the immune system. Other organs such as the liver or kidneys do not appear to be affected to the same extent.

"This close link between inflammation of the heart and brain is a new discovery and an important one, because other studies have shown that an inflammatory response in the brain can promote memory impairment and the development of dementia," explains Professor Bengel. Therefore, closer attention needs to be paid to the impact of heart disease on brain function, and this aspect needs to be taken into account in developing new treatments aimed at improving the healing process. Professors Johann Bauersachs and Kai Wollert from MHH's Department of Cardiology and Angiology and REBIRTH unit on Secreted Factors and Non-Cell-Based Strategies for Cardiac Regeneration supported the project. They both believe that, in the future, it will be possible with anti-inflammatory drugs to

With the small-animal PET camera (from left): Dr James Thackeray and Professor Frank Bengel.

favourably influence not only post-infarction healing but also inflammation in the brain.

"Using our non-invasive molecular imaging techniques, biological mechanisms such as inflammation can be analysed simultaneously wherever they occur in the body – including the heart and brain. And repeated measurements are possible, allowing description of changes over time in a given organism," reports Professor Bengel. The researchers used positron emission tomography (PET), with which they can measure the precise distribution of minute quantities of short-lived radioactive substances (called tracers) in the body.

Their study has been supported by the REBIRTH Cluster of Excellence and by the German Research Foundation-(DFG-) funded Clinical Research Unit KFO 311.

Publication

Thackeray JT, Hupe HC*, Wang Y, Bankstahl JP, Berding G, Ross TL, Bauersachs J, Wollert KC, Bengel FM (*equal contributions). Myocardial Inflammation Predicts Remodeling and Neuroinflammation After Myocardial Infarction. J Am Coll Cardiol. 2018 Jan 23;71(3):263-275.*

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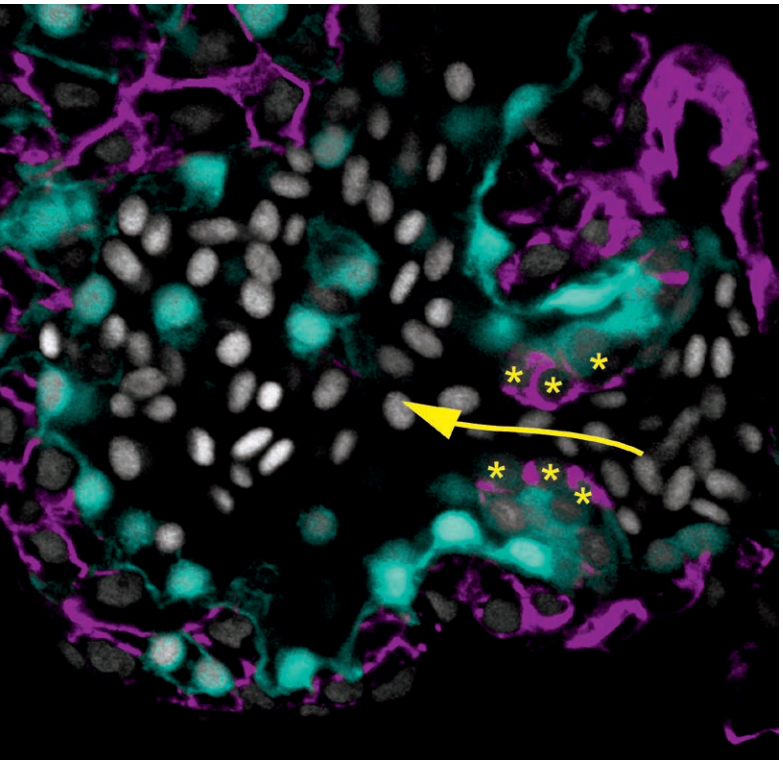


Formation of cardiac valves caused by blood flow (arrow). Blood cells stream into the atrioventricular canal of the zebrafish heart, past the luminal (inner) side of the cardiac valves (asterisks).

How does blood flow regulate heart valve formation?

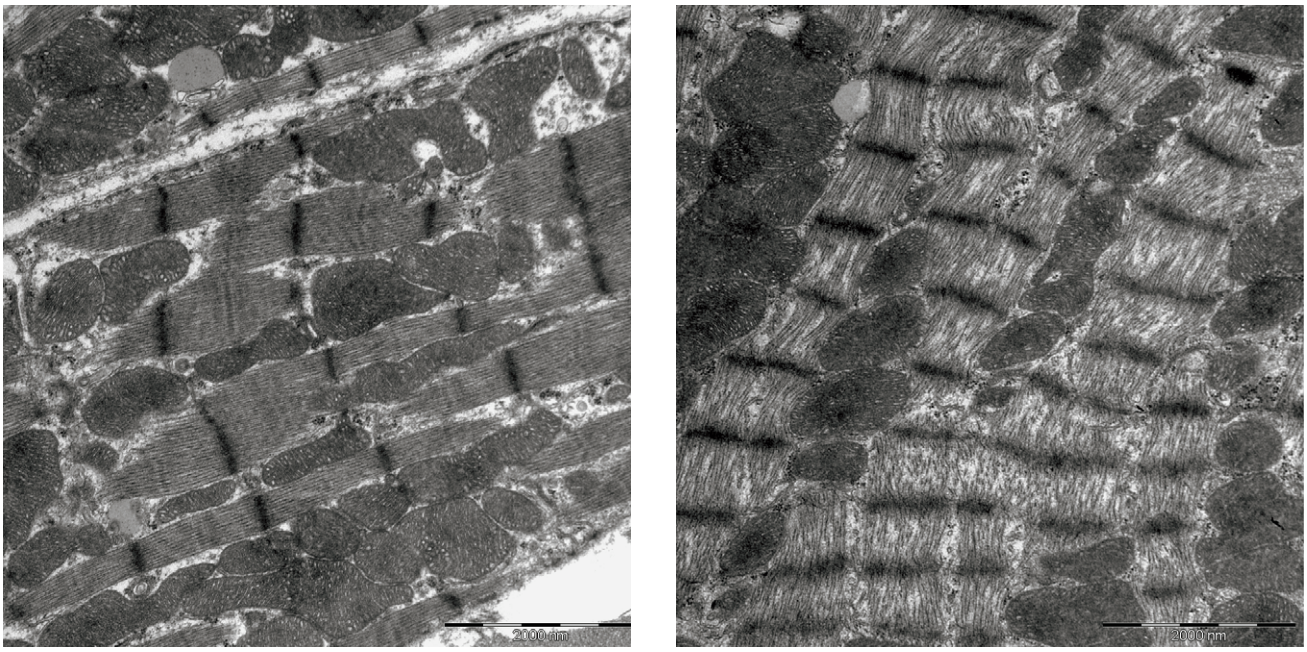
Over the course of an 80-year life, the human heart pumps more than 200 million litres of blood through the body. This feat is crucially dependent on the cardiac valves, which are absent in the early embryonic heart. The rhythmically successive contractions of the ventricles initially generate alternate forwards and backwards flow of the blood. Not until flat structures called cardiac cushions are remodelled into heart valves – valves that move, open and close – does this lead to targeted blood flow, because a backflow of blood is prevented. The heart valves are very simple in structure, with two different sides. Only the cells on the luminal side (i.e. the inner surface) are exposed to the blood flow caused by the heart. The research team led by Professor Salim Seyfried (of the REBIRTH unit on *Zebrafish Cardiovascular Developmental Genetics*) can use microscopic techniques to observe this process in the transparent eggs of the zebrafish.

To determine how the two different sides of the valves form and develop under the influence of the biomechanical forces of blood flow, Professor Seyfried and his team first explored which genes are activated by blood flow during the early development of the zebrafish heart. They found a group of genes that are known in relation to a vascular disease in humans called cerebral cavernous malformations (CCM). The scientists demonstrated that these genes play an important role in the way the cells sense the biomechanical forces of blood flow; they make the cells less sensitive to its effects. When these genes were switched off, heart valves did not form. “We think these genes are especially active in a small part of the cardiac cushion, where they trigger the formation of the abluminal (i.e. outer) side of the cardiac valve. This could prove a focal point for the remodelling of the early cardiac cushion into functional valves,” explains Professor Seyfried (of the University of Potsdam and Hannover Medical School (MHH)). Among the researchers’ aims now are to better understand the mechanism involved, to discover whether the genes are of diagnostic value for congenital heart defects in humans, and, finally, to clarify whether CCM could be caused by altered biomechanical processes in blood vessels. Patients with this condition suffer from vascular adhesions that can lead to haemorrhaging and strokes.

Publication

Donat, S., Lourenco, M., Paolini, A., Otten, C., Renz, M., Abdelilah-Seyfried, S. Heg1 and Ccm1/2 proteins control endocardial mechanosensitivity during zebrafish valvulogenesis. eLIFE 2018; 7:e28939.

Help for heart failure patients after chemotherapy

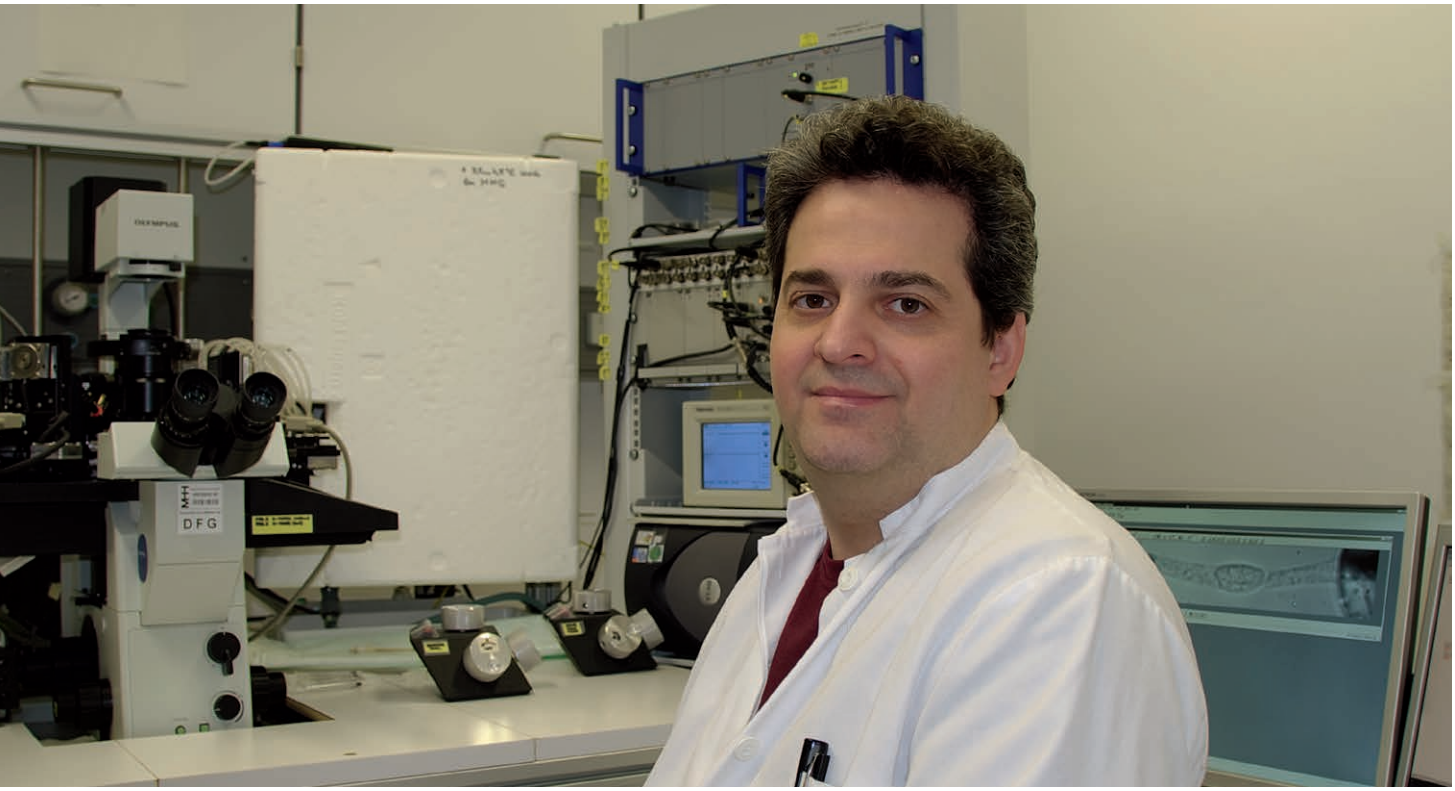


Electron microscope images of heart tissue in mice. The left-hand panel shows healthy tissue. That on the right has been damaged by doxorubicin: the structure of the muscle fibrils is visibly loosened and cloudy.

Although cancer patients often require chemotherapy, this may have strong side effects – for example, the drug doxorubicin may induce heart failure. In tests on mice, researchers at Hannover Medical School (MHH) have now found a potential means of treating this severe adverse reaction. The team led by Professor Thomas Thum at the MHH Institute of Translational and Molecular Therapeutic Strategies brought about successful recovery from heart failure in mice. This involved using gene therapy to insert a gene in the animals that increases the formation of a protein called Quaking. (Chemotherapy has the effect of reducing its production.) Quaking regulates how active circular RNAs are. (These are a specific class of ribonucleic acids.) "We posit that one aspect which these circular RNAs control is cell survival and what is known as programmed cell death," says Professor Thum. He feels treatment with this protein has potential benefits for patients with heart failure in general. The MHH institute that Professor Thum heads is part of the Integrated Research and Treatment Centre Transplantation (IFB-Tx) and is integrated within the REBIRTH Cluster of Excellence.

Publication

Gupta SK, Garg A, Bär C, Chatterjee S, Foinquinos A, Milting H, Streckfuss-Bomeke K, Fiedler J, Thum T. Quaking Inhibits Doxorubicin-Mediated Cardiotoxicity through Regulation of Cardiac Circular Rna Expression. Circ Res. 2018;122(2):246-54. Epub 2017/11/15.



Junior-Professor Bogdan Iorga and the micromechanical setup.

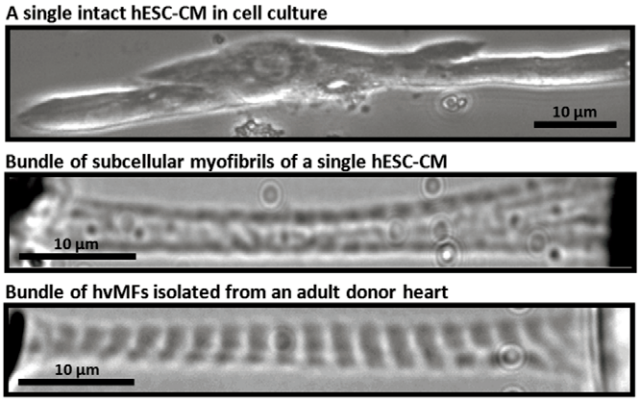
How well do myofibrils contract in human stem cell-derived cardiomyocytes?

Given the essentially unlimited availability of heart muscle cells (cardiomyocytes, CMs) derived from human pluripotent stem cells (hPSCs), they hold great potential for the treatment of cardiovascular diseases by cell transplantation or engineered cardiac tissue, for assessing efficiency and toxicity of pharmacological compounds, or for use as cellular disease models *in vitro*.

However, the stage of maturation of these hPSC-derived CMs (hPSC-CMs) is not easy to determine. "We have to define which aspect we are considering: morphology, gene expression, protein isoform composition or CM function?" explains Junior Professor Bogdan Iorga (REBIRTH unit on Large Animal Models, Institute of Molecular and Cell Physiology). The main function of CMs is to generate force and shorten their length periodically; the subcellular myofibrils contract, thus regulating the pump function of the heart.

To study contractile function of myofibrils within hPSC-CMs and single myofibrils isolated from cardiac tissue, the researchers established a technique based on an atomic-force cantilever used as a nN-sensitive force sensor. Rapid solution changes allow analysis (with very high time resolution) of force kinetic parameters of activation and relaxation of myofibrils and their calcium sensitivity of force. "Using this technique, we compared the contractile function of

myofibrils within human embryonic stem cell-derived CMs (hESC-CMs) differentiated *in vitro* with that of human ventricular myofibrils (hvMFs) isolated from adult donor hearts," says Professor Iorga. "Our experiments showed that certain contraction and relaxation parameters, as well as calcium dependence, differ in the two types of myofibrils, although both – as in the adult heart – contained the ventricular isoform of the cardiac motor protein β-myosin." Analysis of other sarcomeric proteins showed that the



Force generated by subcellular myofibrils was investigated using the micromechanical setup.

hESC myofibrils chiefly had features of human CMs at an early developmental stage. "This explains the functional differences compared with myofibrils from adult hearts," adds Iorga, who has co-authored the relevant study. To further enhance the regenerative potential of *in vitro*-differentiated hPSC-CMs, the scientists are keen to identify the chemo-mechanical factors that induce change in the myofibrils. Moreover, such hPSC-CMs could provide useful models for investigating the contractile function of human CMs during (pre-natal) development of inherited cardiomyopathies or congenital heart defects.

Publication

Iorga B, Schwanke K, Weber N, Wendland M, Greten S, Piep B, dos Remedios CG, Martin U, Zweigerdt R, Kraft T, Brenner B. (2018) Differences in Contractile Function of Myofibrils within Human Embryonic Stem Cell-Derived Cardiomyocytes vs. Adult Ventricular Myofibrils Are Related to Distinct Sarcomeric Protein Isoforms. Front. Physiol. 8:1111. doi: 10.3389/fphys.2017.01111.

Hilfiker-Kleiner joins prestigious academic body

Professor Denise Hilfiker-Kleiner, dean of research at Hannover Medical School (MHH) and leader of a REBIRTH unit, became a new member of Germany's Council of Science and Humanities (WR) with effect from 1 February 2018. Federal president Frank-Walter Steinmeier appointed her to this body for a three-year term, on the strength of a joint nomination by the German Research Foundation (DFG), the Max Planck Association, the German Rectors' Conference, the Helmholtz Association, the Fraunhofer Society and the Leibniz Association.

The WR is the most important science policy advisory body in the country, providing guidance to government at both national and federal-state level. Its focus is the policy environment for research, teaching and studying in Germany.

Professor Hilfiker-Kleiner has been MHH's dean of research since 2013. In this capacity, she advises the University's Presidium (governing body) and Senate on research issues, chairs meetings of the Research Committee (whose members prepare decisions and recommendations on research matters) and also chairs various boards of trustees.

New strategic partnerships initiated

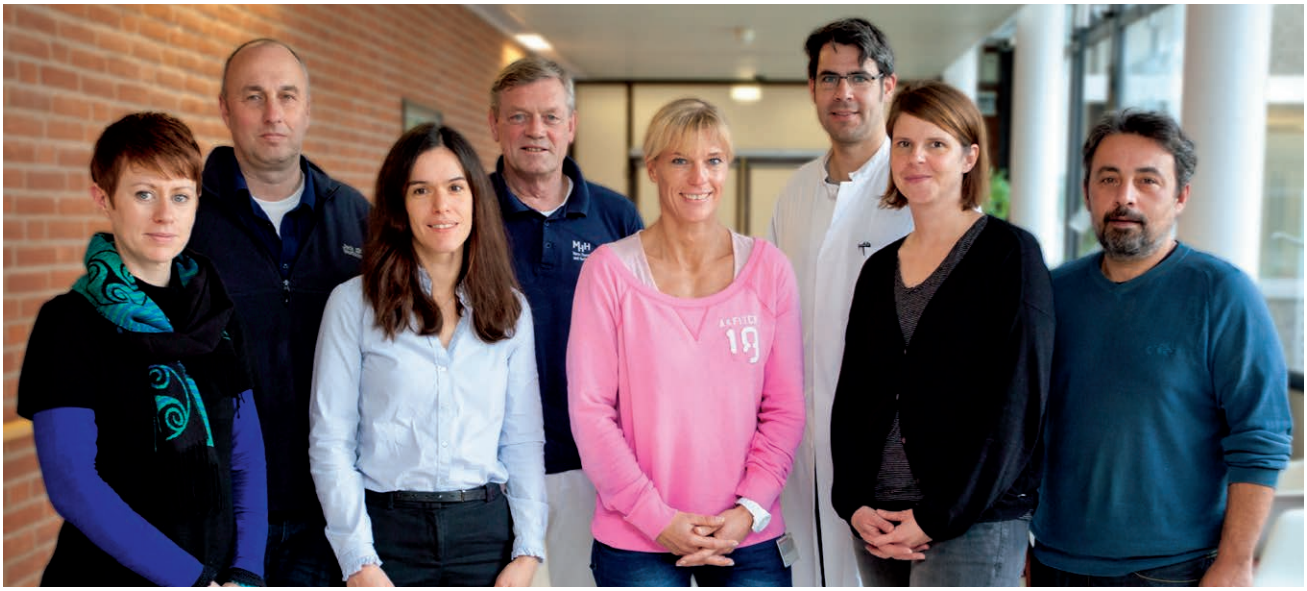
For an internationally renowned centre of regenerative medicine such as the REBIRTH Cluster of Excellence, strategic international partnerships have a measurable and lasting impact on their success. Over the past 18 months, therefore, the Cluster has, based on an internationalization plan, initiated and given shape to formal agreements with several world-class centres for regenerative medicine. Strategic cooperation partners include the Harvard Stem Cell Centre, two Toronto University programmes – the Medicine-by-Design Program (MdB) and the Translational Biology and Engineering Program (TBEP) – the Guangzhou Institute for Biomedicine and Health (GIBH, including the Max Planck-GIBH Joint Center for Regenerative Biomedicine, under H. Schöler) and the Kyoto Graduate School of Medicine (Faculty of Medicine). The partner institutions have signed Letters of Intent that will pave the way for detailed collaborative agreements. To this end, the Cluster is also in contact with the Vascular and Regenerative Medicine Centre in Leiden.

The strategic partnerships of the REBIRTH Cluster of Excellence are based on existing collaborations, established personal contacts and a strong mutual interest in maximizing synergies between the participating organizations. Research, translation and clinical application activities will benefit from these partnerships. A further aim is the boost that international collaborative settings will give to the careers of the researchers and clinicians involved, as well as to the future potential of the Cluster itself.

Save the Date: 10 years of CELLS

From 14 to 16 November 2018, the Centre for Ethics and Law in the Life Sciences (CELLS) at the Leibniz Universität Hannover will be celebrating its 10th birthday! We will be marking the occasion with a stimulating series of events in the Leibnizhaus, the guest house of Hannover's higher-education institutions.

To find out more about this event, visit
https://www.cells.uni-hannover.de/



How are artificial lungs implanted?

Researchers involved in the project (from left): Nina McGuinness, Professor Wim Wolkers, Dr Constanca Ferreira de Figueiredo, Professor Axel Haverich, Dr Bettina Wiegmann, Dr Christian Kühn, Dr Ruth Olmer and Dr Sotiris Korossis.

REBIRTH researchers involved in nationwide collaborative programme

For people with certain serious lung conditions, a lung transplant is the only curative treatment option. However, only a small proportion of patients can receive this treatment, as there are fewer and fewer organ donors but more and more individuals who require a donor organ. The German Research Foundation (DFG) is now supporting a priority programme called 'Towards An Implantable Lung' (SSP 2014) to the tune of 12.6 m euros spread over six years. Scientists at Hannover Medical School (MHH) who are participating in it will be receiving 1.4 m euros for their research projects over the first three years. Rheinisch-Westfälische Technische Hochschule (Aachen University, (RWTH)) and the Universitätsklinikum Aachen (university hospital) are coordinating the programme, in which two other university hospitals – those at Regensburg and Tübingen – are also involved. The researchers on this priority programme aim to develop a bio-artificial lung (BA) for permanent use as an alternative treatment to lung transplantation. "Whereas considerable progress with artificial hearts has been made in recent years, the development of an (urgently needed) artificially implantable replacement for the lung – a far more complex organ than the heart – is still in its infancy," says Professor Axel Haverich, director of MHH's Department of Cardiothoracic, Transplantation and Vascular Surgery (HTTG), coordinator of both the REBIRTH Cluster of Excellence and the Hannover-based part of the programme. His department transplants more donor lungs than any other centre worldwide.

The 'bio-artificial' lung

An assist device called the extracorporeal membrane oxygenator (ECMO) serves as the basis for the 'bio-artificial lung', which can provide short-term functional support or replacement for the lung. Long-term use is not yet an option, however, as one of the body's reactions to the ECMO's plastic surfaces is the formation of blood clots. To prevent this, researchers on the priority programme are working on an interdisciplinary basis, exploring various strategies to enhance this support system. For example, the investigators aim to optimize the compatibility of these plastic surfaces with the blood by means of cell seeding, and to improve blood flow conditions and the miniaturization of the system, in order to achieve both long-term use and the bio-artificial lung's full implantation in the body.

Decellularized heart valves:

ARISE trains medics

A training workshop organized by collaborative research alliance ARISE was held in Barcelona on 17 November 2017. ARISE stands for *Aortic Valve Replacement using Individualised Regenerative Allografts: Bridging the Therapeutic Gap*, and the project has been conducting an EU-funded clinical trial since early 2015. In this study, of which Hannover Medical School (MHH) is the lead institution, physicians are exploring a new replacement allograft for the aortic valve: one that is not rejected and is more durable. Five other major heart centres in Europe are also participating. It was envisaged that 120 patients would be treated in four years.

In the workshop, those in charge of the study presented the new valve replacement – not only in theory but also in practice – and provided an update on the project.

"We were pleased to be able to report at the workshop that, after only two years of the trial, 99 patients had received a decellularized cardiac valve. Now – in the summer of 2018 – it's already as many as 127 individuals, of whom 115 are included in the study," says Professor Samir Sarikouch, head of Clinical Trials at MHH's Department of Cardiothoracic, Transplantation and Vascular Surgery (HTTG).

The workshop was attended by 35 young medical students, cardiologists and surgeons from throughout Europe, as well as tissue bank workers, transplant coordinators and representatives of patients' organizations. The highlight was a practical component in which, guided by professionals, the participants learned how to suture – into pig's hearts – the decellularized valves (both pulmonary and aortal) that spinoff Corlife GbR had provided for training purposes. In this way they learned how to handle the 'product' correctly. Two tutorial videos, in which surgeons commentate as they operate on the first ARISE patients in Hannover and Barcelona, were used by way of preparation. They enabled those undergoing instruction to observe the surgical techniques in detail beforehand.

Left: Dr. Quintana demonstrating the operation technique to non-surgeons.
Right: Participating surgeons during the wet lab session.

To find out more about the workshop, follow this link: www.arise-clinicaltrial.eu/fileadmin/redakteure/ARISE_Login-Bereich/ARISE_D6.3_final.pdf

B!G B4NG:

REBIRTH sets pupils challenging task

The REBIRTH Cluster of Excellence took part in this winter term's B!G B4NG Challenge, a competition for school pupils (previously called *Club Apollo 13*) organized by *uniKIK* at the University of Hannover (LUH).

The B!G B4NG Challenge is a contest for pupils in Year 9 and above throughout northern Germany. In groups of three to five, they work on tasks – for each of which around four weeks are allocated – drawn from different disciplines. They had 30 different tasks to choose from.

The REBIRTH unit on *Biomedical Photonics for Regeneration Studies* set the following task: 'Interdisciplinary excellence: what physics and biology can teach us about regenerative mechanisms'. Pupils were asked to explore both theory and practice in areas such as the principles of microscopy, using 'household agents' to isolate DNA, and various approaches to cloning. 30 groups worked on the task. "When going through the pupils' solutions, it became evident that pupils have a lot of fun with interdisciplinary topics like those investigated in REBIRTH. Many groups submitted great solutions, even though parts of the problem were deliberately made harder for school pupils to solve," says Dr Stefan Kalies, who heads the REBIRTH unit involved. "We'd definitely be keen to set another task drawn from the REBIRTH environment in September as well," he adds. "If this appeals to anyone, they're welcome to get in touch with me."

This is a further successful cooperative effort between REBIRTH and *uniKIK* (another being the 'Autumn Academy for Teachers'.

Contact: Stefan Kalies: kalies@iqo.uni-hannover.de

Further information about the B!G B4NG Challenge is available (in German) www.studienberatung.uni-hannover.de/bigbangchallenge.

REBIRTH successful in competition for young researchers

Among the younger researchers in 'Jugend forscht', a federal-state-wide competition for young researchers, Philipp Melk and Lennart Jathe came second in the Biology category. They are both Year 6 pupils at a Hannover secondary

Budding ant researchers: Philipp Melk (left) and Lennart Jathe.

school (Kaiser-Wilhelm- und Ratsgymnasium) and, in early April, were commended for research findings from their project, 'Off-form FORMicidae – do ants love junk food?' Back in February, they had impressed the jury at the regional heats for this contest in Hannover, thus qualifying for the federal-state-wide contest. "As several hundred projects are entered for the regional competitions, this is a terrific achievement and shows that Hannover Medical School (MHH) is successful at supporting young scientific talent from a very early stage," says Professor Meike Stiesch. As head of the 'Biofabrication for NIFE' research alliance, and in conjunction with the REBIRTH Cluster of Excellence, she assisted with the purchase of an A.N.T.S 'Hands-on Experimental Kit' from Johannes Gutenberg University Mainz for the 'Jugend forscht' project.

The dynamic young research duo explored how a high-fat diet affects the regenerative capacity of ants. In a long-term feeding experiment they showed that, although ants fed a fat-rich diet were larger than those given normal food, they did not produce as many offspring. Buoy-ant with success, the two 'ant boys' have a new goal for the coming year: to follow up by investigating whether increased 'exercise' can improve the insects' survival.

Cooperation does matter

In regenerative medicine and tissue engineering, multidisciplinary research is playing an increasingly important role in providing complementary insight into various health-related phenomena.

Vitalii Mutsenko (right) discussing findings and further planning of experiments with Professor Damijan Miklavčič (left).

From August to November 2017, Vitalii Mutsenko, (REBIRTH unit on Cell Protection Technology, Institute for Multiphase Processes, Leibniz Universität Hannover), took part in two outgoing visits to Slovenia and Greece sponsored by the IP@Leibniz programme.

''In going to the Laboratory of Biocybernetics at the University of Ljubljana in Slovenia, I wanted to take a look at cell and tissue cryopreservation from the electrical engineering point of view. Indeed, work on electroporation of stem cells to enable their *xeno*-free cryopreservation proved very stimulating and productive, and I am looking forward to establishing this technology at our Institute and strengthening our cooperation with Slovenian partners," says Vitalii Mutsenko, a student on the Ph.D. programme in Regenerative Sciences.

Prompted by a REBIRTH Special Lecture given by associate professor Dr Elias Anastassopoulos (Department of Agricultural Engineering Technologists, TEI of Thessaly,

Vitalii Mutsenko (right) with Professor Elias Anastassopoulos, collecting leaves from Hippóphae rhamnoídes.

Greece), he visited Anastassopoulos' Lab to study cryoprotective agents of natural origin using a technology, which is based on infrared video recording of multiple ice nucleation events occurring during freezing of tissue-engineered scaffolds in various culture plates and cryobags. ''I believe that the solution to the problem of organ cryopreservation will be adopted from the natural world in which many organisms can survive very low temperatures", says the biochemist.

Fiedler: one of the first eight graduates

On 26 October, the TRAIN Academy produced its first graduates. It offers a part-time professional-development programme for working researchers. After two years of weekly lectures and practical units, eight participants are now certified experts in translational science. REBIRTH researcher Dr Jan Fiedler is among them. "What I especially appreciate about the TRAIN Academy is that this is a regional opportunity which enables me to network here in the local area," says Fiedler, who heads a research unit at the Institute of Molecular and Translational Therapeutic Strategies (IMTTS). "I'm at a stage in my career when I need to take stock and move forward, but professional-development opportunities for post-docs are few and far between. The TRAIN Academy is a great way of making contacts in the region, exploring other research environments than purely academic ones, and advancing one's career by upgrading one's skills."

Poster prize for Olbrich

In collaboration with the Baylor College of Medicine in Houston, Texas, the team from the REBIRTH unit on *Regenerative Immune Therapies Applied* – headed by Professor Renata Stripecke – designed and constructed retroviral vectors expressing chimeric antigen receptors (CARs) targeting the CMV glycoprotein B (gB). Using these vectors, medical student Henning Olbrich generated gB-CAR T cells as part of his structured doctoral programme. These engineered T cells recognize and kill CMV-infected cells *in vitro*. This antiviral effect could be further enhanced upon combination of gB-CAR T cells with dendritic cells engineered to express the gB protein on the cell surface. Pilot experiments using humanized mice infected with CMV showed promising effects for this combination immune therapy. For this work, Olbrich was awarded a poster prize during the German Centre for Infection Research's (DZIF) 2017 Annual Meeting.

Gupta receives award

The Hans-Heinrich Niemann-Gedächtnispreis, a memorial prize worth 2,500 euros endowed by Professor Teruko Tamura-Niemann, has been awarded to Shashi Kumar Gupta, Ph.D., of MHH's Institute of Molecular and Translational Therapeutic Strategies. With the institute's director Professor Thomas Thum (REBIRTH unit on *miRNA in Myocardial Regeneration*) he co-led a research team that set in motion a novel heart attack treatment primarily for older patients. The investigators discovered that inhibiting a specific ribonucleic acid chain (microRNA 22) has a twofold positive effect. Firstly, it stops abnormal heart growth, thus preventing cardiac insufficiency. Secondly, it facilitates the cells' own 'refuse collection' process by making waste products easier to break down and remove.

Gupta S.K., Foinquinos A., Thum S., Remke J., Zimmer K., Bauters C., de Groote P., Boon R.A., de Windt L.J., Preissl S., Hein L., Batkai S., Pinet F., Thum T. Preclinical Development of a MicroRNA-Based Therapy for Elderly Patients With Myocardial Infarction. J Am Coll Cardiol 2016; 68:1557.

Grothausmann wins Paper of the Year award

Dr Roman Grothausmann (of the REBIRTH unit on *Quantitative Microscopy in Regeneration* at MHH's Institute of Functional and Applied Anatomy) won the 2017 Paper of the Year award conferred by the *American Journal of Physiology – Lung Cellular and Molecular Physiology*. The publication was on the 3D reconstruction and virtual endoscopy of the capillary system in the lung (http://osf.io/gbsns/).

Grothausmann R., Knudsen L., Ochs M., Mühlfeld C. Digital 3D reconstructions using histological serial sections of lung tissue including the alveolar capillary network. Am J Physiol Lung Cell Mol Physiol 2017; 312:L243.

Wollert: Honorary award for cardiology lecture

Professor Kai Wollert (REBIRTH unit on *Secreted Factors and Non-Cell based Strategies for Cardiac Regeneration*, MHH Department of Cardiology and Angiology), was presented with the Honorary Award 2018 Lecture on Basic Science at the 84th Annual Meeting of the German Cardiac Society (DGK). He was awarded this prize, worth 1,500 euros, for his lecture entitled 'Infarct healing as a therapeutic target: from cells to proteins and small molecules'.

EU funds REBIRTH researchers

PROJECT RECOMB • The team led by Professor Axel Schambach and Dr Michael Rothe – of the REBIRTH unit on *Regenerative Gene Therapy* based at the Institute of Experimental Haematology – is participating in a five-year project called RECOMB. It is receiving some 560,000 euros in funding for this purpose. Within this consortium, the scientists are working to develop gene therapies for severe combined immunodeficiency, a serious immune-system dysfunction that affects sufferers from birth. The researchers are developing methods aimed at replacing defective genes in these patients with 'correct' genes. This Hannover Medical School (MHH) team is creating gene vectors: molecules into which the desired genes are incorporated. These are introduced into blood-forming stem cells for subsequent transfer into the patient's cells. This group is also developing new techniques for verifying the safety of these gene vectors.

PROJECT REPO-TRIAL • Many already approved medications may potentially also be used to treat other conditions, as these drugs often influence fundamental and common mechanisms of disease. REPO-Trial, a collaborative project set up for six years, has set itself the task of assessing just such medications (i.e. those already approved for other indications) as to their effect on cardiovascular illness. In a subproject funded to the tune of 550,000 euros, the team headed by professors Johann Bauersachs and Daniel Sedding – of the REBIRTH unit on *Vascular Remodeling und Regeneration* within MHH's Department of Cardiology and Angiology – is investigating possible additional benefits of established medicines in patients with heart failure.

Ph.D. Programm Regenerative Sciences

Doctorates obtained in January & June 2018

On 12 January, all that lay between five young women and the successful conclusion of their doctoral thesis was their oral defence. However, for Professor Ulrich Martin, chairperson of the Ph.D. programme in Regenerative Sciences and deputy coordinator of the REBIRTH Cluster of Excellence, there were still just over five weeks until the submission deadline for the REBRTH 3 proposal. Despite the intensive paperwork this entailed, he took the time at the end of the day to address some personal words to the new post-docs during their graduation ceremony.

When the final examinations were next taken in June, the REBIRTH 3 proposal had already been presented to the international review board in Frankfurt, and there was great relief and elation on the evening of 1 June. We wish all of the graduates – and the Cluster – all the very best for the future!

Students who obtained their doctorate in January

- **Lena Engels** (Dr. rer. nat.) *Genetic Engineering of the HLA Repertoire of Human Induced Pluripotent Stem Cells to Overcome T Cell and NK Cell Response after Allogenic Cell Therapy* Supervisor: Professor Ulrich Martin, Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), Hannover Medical School (MHH)
- **Melina Heise** (Dr. rer. nat.) *Morphogenetic Control of Zebrafish Cardiac Looping by Bmp Signaling* Supervisor: Professor Salim Seyfried, Institute of Molecular Biology, Hannover Medical School (MHH)
- **Laura Korte** (Dr. rer. nat.) *Impact of miRNA-146a on Vascular Remodeling Processes* Supervisor: Professor Daniel Sedding, Department of Cardiology and Angiology, Hannover Medical School (MHH)

front, from left to right: Laura Korte and Melina Heise; back, from left to right: Lena Engels, Hanna Wolling (née Möller) and Madline Schubert (née Götz).

from left to right: Bulat Sydykov, Monika Szépes, Dorothee Eicke (née Heinemann) and Tom Wahlicht.

- **Madline Schubert** née Götz (Dr. rer. nat.) *Generation of Disease-specific hiPSCs and Development of Transgenic Reporter Cell Lines for Cystic Fibrosis Disease Modelling and Drug Screening* Supervisor: Professor Ulrich Martin, Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), Hannover Medical School (MHH)
- **Hanna Wolling** née Möller (Dr. rer. nat.) *Establishment of a BSA-free Medium Supplement Enabling Secretome Analysis during Human Pluripotent Stem Cell Cardiomyogenesis as well as Xeno-free Differentiation into Definitive Endoderm* Supervisor: apl. Professor Falk Büttner, Institute of Clinical Biochemistry, Hannover Medical School (MHH)

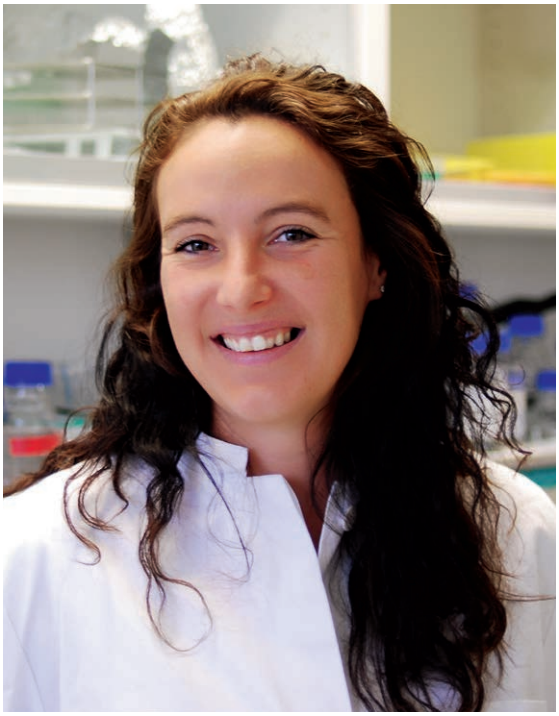
Students who obtained their doctorate in June

- **Sandra Baus** née Weinreich (Dr. rer. nat.) *Generation and Enrichment of Airway Basal and Club Cells from Human Induced Pluripotent Stem Cells Utilizing Double Transgenic Reporter Lines* Supervisors: Dr. Ruth Olmer & Professor Ulrich Martin, both Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), Hannover Medical School (MHH)
- **Dorothee Eicke** née Heinemann (Dr. rer. nat.) *Large-scale Production of HLA-silenced Megakaryocytes and Platelets for Universal Application* Supervisor: PD Dr. Constanca Figueired
- **Bulat Sydykov** (Dr. rer. nat.) *Macromolecular Stability of Biological Glasses* Supervisor: Professor Willem F. Wolkers, Institute of Multiphase Processes, Leibniz Universität Hannover
- **Monika Szépes** (Dr. rer. nat.) *Investigation of the Role of Pericytes in the in vitro Model of Bioartificial Tissue Formation from Human iPSC-derived Cardiovascular Cell Types* Supervisor: PD Dr. Ina Gruh, Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), Hannover Medical School (MHH)
- **Tom Wahlicht** (Dr. rer. nat.) *An in vitro Liver Zonation System Based on Controlled Modulation of Wnt-signalling in Hepatic Cells* Supervisor: Professor Dagmar Wirth, Model Systems for Infection and Immunity, Helmholtz Centre for Infection Research (HZI)

Ph.D. Programme *Regenerative Sciences*

Who is Who

***Friederike Philipp** (35), started her Ph.D. in 2014, Germany, REBIRTH Unit on* Preclinical Safety and Toxicology *in cooperation with MHH-Institute for Experimental Haematology*



What are you working on and why?

My Ph.D. project is a collaborative effort between the Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM) and the Institute of Experimental Haematology at Hannover Medical School (MHH). Both institutes are REBIRTH members and have a particular interest in the development and risk assessment of cellular and gene therapies to treat immune conditions. The focus of my project is on establishing and characterizing an embryogenesis model in which, using stem cells as the base material, we can represent and investigate early human haematopoiesis. The deeper understanding gained will help to improve cellular and gene therapies in the future.

Why did you decide to enrol in the Ph.D. programme in Regenerative Sciences?

Having studied in Düsseldorf, I felt the Ph.D. programme – with its lectures – would be an ideal way to get an in-depth overview of the different work groups at MHH. I was also impressed by the strong thematic links with clinical practice. In various tutorials, the programme provides an opportunity to get alongside specialist physicians in their respective wards and thus to see research being practically applied. In addition to well-structured supervision for one's Ph.D. project, students on the programme can also attend many seminars and workshops enabling them to develop professionally. The ones I've got the most out of so far have been project management and scientific presentation techniques.

What do you like about Hannover Medical School?

MHH is a great place for stem cell research. Many groups at MHH and nearby institutes do excellent research work in a variety of fields and are strongly interlinked. This is crucial, since the more complex our findings and the resulting methods are, the more important are mutually complementary collaborations with 'cross-pollination' of expertise and modern equipment. Being integrated within two different institutes has enabled me to benefit in many ways from this excellent cooperation.

What makes Hannover different from your home town?

I come from the Markgräflerland region in Germany's warm southwest. Having opted for Hannover, I initially met with scepticism from friends: "They only speak High German there – when they speak at all, that is", and "It's so cold in the North," were the commonest objections. But right from the start, beautiful Maschsee Lake, the vast urban forest of Eilenriede and the very well developed network of cycle paths persuaded me I'd chosen a great place to live. I love the calmness, straightforwardness and humour of the people here.

What three things you would take to a desert island?

A good book by Hakan Nesser or Jussi Adler Olsen, a case of wine from home – which means a corkscrew would also be a must!