Dear Colleagues and Friends,

In 2017 the IZKF started in a new phase. After extensive discussions and after achieving a broad consensus within the Faculty of Medicine it was decided to restrict the main IZKF funding on only two joint research projects with a very high potential for secondary major external funding such as a DFG “SFB”. The selection process was not easy. Each of the major research fields of the Faculty had to come up with one proposal except for the cardiovascular research field because of their successful application of a DFG TR-SFB. From the other four fields proposals were sent to external reviewers. The reviewers were selected according to their expertise in the field by a group of three neutral and responsible faculty members (the Dean, the Speaker of the IZKF and the Dean for Research). In contrast to earlier review processes this time the reviewers were paid. And also in contrast with earlier times we did not organize an on-site review meeting. We did not elect an advisory board or a supervisor to manage the review process. We all felt that the management of the process is our own responsibility and as one of our former advisors told us, it is our money, it is our strategy, it is our decision.

After receiving in-depth reviews in a very good quality the IZKF steering committee decided that the best reviews were given for the project in Oncology namely “Mesenchymal interactions and fibrogenic signaling in cancer development” coordinated by Professor Weiskirchen and in Inflammation namely “The gut liver axis” coordinated by Prof. Hornef. These projects started in time.

The currently active and successful IZKF research groups of Dr. Liehn in Cardiovascular Research, Dr. Strnad in Inflammation, Dr. Haiss in Neuroscience and Dr. Costa in Bioinformatics are slowly coming to the end of their funding period and the application process started to establish two new groups. These new groups should have their focus in Medicine & Technology, Cardiovascular Research or in Translational Neuroscience. Dr. Haiss left the Faculty and will continue his work at the Institute Pasteur in Paris, Drs. Liehn and Strnad will stay in our Faculty and Dr. Costa is now a professor for Computational Genomics in our Faculty.

We have also started funding small and short projects and we continued our funding of IZKF Core Facilities. In addition to the established Core Facilities we started a new core for FACS analysis and sorting coordinated by Professor Pabst. This unit is already running.

The former IZKF board is now the IZKF steering committee. This label better describes the responsibility of this group of people. Each of the major research areas is represented in this group, the Core Facilities are represented and to improve the cooperation with the institutes of engineering and natural sciences we elected Professor Marc Spehr from the Department of Biology as the RWTH liason professor.

Many decisions have been made over the past years transforming “IZKF Biomat” as a kind of institute with a large in-house budget to “IZKF Aachen” as a professionally managed in-house funding agency and research management structure supporting the strategy and the goals of the Faculty of Medicine at RWTH Aachen University. I had the opportunity to go along with this process with many friends and colleagues within the IZKF board and the Faculty, with the administrators and managers in the IZKF Office and also with the researchers within projects and the core facilities. It was not always easy to find a consensus in difficult questions and sometimes it was not possible to find a solution which was right for everyone. But at the end of the day the best decisions have to be made to provide a platform for research as good as possible.

I think this goal was achieved but we can and we will go beyond this point. I really feel privileged being able to work together with so many bright persons. Together we can further improve the performance of IZKF Aachen and I am looking forward to another exciting year.

Sincerely,

Prof. Dr. Peter Walter

on behalf of the Steering Committee of IZKF Aachen
The project leaders are responsible for the content of their reports and for the information on their external funding, publications, etc.
The IZKF in 2017

External evaluation process for the Core Facilities

The Core Facilities were evaluated for the first time according to the newly defined processes. Each facility was evaluated by two external reviewers with experience in the Core Facility management. The Brain Imaging, Genomics, Immunohistochemistry, Confocal Microscopy, Proteomics and Two-Photon Imaging facilities additionally received a personal onsite visit from one of their reviewers including a consultation with the head of the facility, the supervisory board and the research coordinator. The comprehensive, individual reviews contained constructive criticism with suggestions for improvement, and also certified the facility’s technical equipment with a good to very good rating. A broad suggestion was the implementation of a software tool for managing the facilities in order, for example, to illustrate economic efficiency and capacity utilisation more objectively.

Further funding for existing Core Facilities and a new Flow Cytometry Facility

After the external reviews, the steering committee confirmed further funding for all Core Facilities for another three years according to their applications. A new Flow Cytometry Facility has been established under the IZKF organisational umbrella in response to faculty requirements for cell sorting and external recommendations. The significance of the central services for faculty research was reaffirmed: It is increasingly important to promote cooperation between groups within the faculty which make use of the same equipment and techniques.

Investments and Core Facilities development

To meet current demands, a second-hand mass spectrometer was acquired for the Proteomics Facility, in addition to the existing mass spectrometer, with funds from the Dean’s Office and the IZKF.

Upon recommendation from an external reviewer to introduce a professional Core Facility Management System, the research coordinator organised an online presentation of the PPMS software from Stratocore. The software would provide the Core Facility Manager not only with an automated booking system, but also with the possibility to create reports about equipment users and user behaviour i.e. methods. Internal cost allocations would also be created automatically as well as mailing lists for improved communication with users. The software is currently being tested.

New information flyers were created for all Core Facilities to further promote their services and methods within the Faculty of Medicine. The information available online at www.izkf-aachen.de was also updated.


Prior to submitting their applications, the Speakers for the Research Focus Areas sent the following declarations of intent thereby referring to the external funding objectives:

<table>
<thead>
<tr>
<th>Research Focus Area</th>
<th>Project Title</th>
<th>Coordinator</th>
<th>Number of Subprojects</th>
<th>External Funding Objective</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medicine and Technology</td>
<td>Forces in 4D: Cellular strategies in coping with mechanical stress</td>
<td>Leube</td>
<td>9</td>
<td>Research Training Groups, CRCs</td>
</tr>
<tr>
<td>Clinical Neurosciences</td>
<td>Sensory nerve dysfunction in small fiber neuropathies</td>
<td>Weis</td>
<td>7</td>
<td>DFG Clinical Researcher Groups</td>
</tr>
<tr>
<td>Inflammation and Consequences</td>
<td>The gut liver axis – functional circuits and therapeutic targets in health and disease</td>
<td>Hornef</td>
<td>15</td>
<td>DFG Collaborative Research Centre</td>
</tr>
<tr>
<td>Oncology</td>
<td>Mesenchymal interactions and fibrogenic signaling in cancer development: Molecular mechanisms in solid and hematologic neoplasia</td>
<td>Weiskirchen</td>
<td>11</td>
<td>CRC or CRC/IFR</td>
</tr>
</tbody>
</table>

The working group consisting of the IZKF Speaker, the Deputy IZKF Speaker, the Dean and Vice-Dean for Research assigned three external reviewers per joint proposal. The paid reviewer evaluated the applications according to scientific excellence, the ability to produce the intended measures and the proposal’s potential for acquiring third-party funding. The evaluation criteria were largely the same as DFG criteria. After receipt of the anonymous, written reviews, the working group reconvened to intensively consider the evaluation and to develop a funding proposal for the steering committee. The steering committee also provided their assessment of funding ability and prioritisation. This resulted in a clear conclusion very similar to that of the working group. The steering committee thus unanimously decided to fund the following two joint projects for a further three years from 2018.

- “Mesenchymal interactions and fibrogenic signaling in cancer development” (Oncology)
- “Gut Liver Axis” (Inflammation and Consequences), with the following conditions: The CRC application should be submitted to the DFG in September as planned.
  - IZKF funding will be discontinued when the CRC funding begins.
  - IZKF funding will be discontinued if the preliminary application receives a C rating from the DFG.
1.1 | The IZKF in 2017

Announcement and Call for Proposals for the “Advanced Program”
As part of an advanced program, funding for at least five individual projects was announced for a maximum period of one year. The funding per project amounts to a maximum of € 20,000 exclusively for consumables. Individual projects can be proposed regardless of the research focus area, in order to test a hypothesis or develop ideas. The program is targeted to “advanced” applicants who can no longer apply for START funds i.e. working group leaders and upwards.

Announcement and Call for Research Groups
The IZKF invited applications for two Research Group Leader Positions with recognized expertise in the research areas: Medicine and Technology, Cardiovascular Research and Clinical Neurosciences. The aim of the call is to identify scientific topics to strengthen the mentioned Research Focus Areas and to invite external suitable candidates to apply for the group funding.

The IZKF will fund - from 1.1.2019 - two Research Groups with a funding period of three years, with the possibility of a three-year extension after positive external evaluation.

Each group will be equipped with an annual budget of € 200,000 (including the position of the research group leader).

The Research Groups will have to be supported by at least one department of the Faculty of Medicine, that will provide the group with basic facilities.

Election of the Speaker and the Deputy Speaker of the IZKF by the Faculty Board, October 9th
During the faculty meeting on October 9th, Prof. Peter Walter was re-elected as the Speaker of the IZKF by the Faculty Board for another three-year period. Prof. René Tolba was elected as the Deputy Speaker for the next three years.

Prof. Spehr was unanimously elected as the Representative of the Faculties of Engineering and Natural Sciences.

Election of the steering committee
The Speakers for the faculty research focus areas were requested to nominate members for the new IZKF steering committee. A Focus Area Coordinator and Deputy were proposed for each research focus area. Focus Area Coordinators will now no longer receive IZKF funding themselves.
Core Facility Managers were also requested to select Deputies from among their colleagues to be assigned to the steering committee.

The newly elected steering committee consists of:

IZKF Speaker
Prof. Dr. R. Tolba

IZKF Deputy-Speaker
Prof. Dr. P. Walter

Focus Area-Speaker
Prof. Dr. R. Tolba

Focus Area Coordinator for Medicine and Technology
Prof. Dr. H. Fischer (SP 1)

Focus Area Coordinator for Cardiovascular Research
Prof. Dr. N. Marx (SP 2)

Focus Coordinator for Clinical Neurosciences
Prof. Dr. U. Habel (SP 3)

Focus Coordinator for Inflammation and Consequences
Prof. Dr. M. Müller (SP 4)

Focus Area Coordinator for Oncology
Prof. Dr. B. Lüscher (SP 5)

Representative of the Faculties of Engineering and Natural Sciences
Prof. Dr. M. Spehr

Core Facilities Representative
Prof. Dr. G. Müller-Neven
Day of Medical Research – Best Paper Award, December 15th
On December 15th, the IZKF organized the annual Day of Medical Research in collaboration with the Faculty of Medicine Dean’s Office. This well-attended event has been a very positive experience with a stimulating and interactive atmosphere during the last years. Prof. Dr. Dirk Halle of the TU München presented “Microbiome Signatures: Specificity and function of complex ecosystems”. The author of the “Best Paper 2017” was awarded with a prize of €1,000, sponsored by Bayer Health Care. The two winners were Anne Schneider and Beate Karges (Internal Medicine III) with the following publications:


A lecture by Anne Schneider concluded the first part of the day. Thereafter, all seven joint research projects funded by IZKF were presented by their coordinators in a short presentation. The projects funded by START presented their concepts and results in a poster session.
Funding Measures in a Nutshell

Project Funding

- 44 projects (46% of the total budget during the report period)

Supporting of Junior Scientists

- 4 Research Groups (14% of the total budget during the report period)

Core Facilities / Laboratory

- Core Laboratory
- Genomics Facility
- Immunohistochemistry Facility
- Confocal Microscopy Facility
- Brain Imaging Facility
- Two-Photon Imaging Facility
- Transgenic Service
- Proteomics Facility
- Flow Cytometry Facility (36% of the total budget during the report period)

4% of the total budget during report period was expended for the scientific coordinating office, central costs, structural measures and investments.

Funding and Financial Overview

In 2017, the IZKF received €3,175,380 from the State Grant for Research and Teaching as fixed in the faculty budget. It also received a faculty grant of €1,790,772. The total budget amounted to €4,972,152. An additional sum was kept available by the faculty, in case the projects needed budget rests from earlier years.

Expenses

<table>
<thead>
<tr>
<th>Budget 2016</th>
<th>€4,972,152</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Expenses 2016</td>
<td>€4,943,218</td>
</tr>
<tr>
<td>Projects</td>
<td>€2,271,997</td>
</tr>
<tr>
<td>Medicine and Technology</td>
<td>€499,417</td>
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<tr>
<td>Cardiovascular Research</td>
<td>€322,306</td>
</tr>
<tr>
<td>Clinical Neurosciences</td>
<td>€423,544</td>
</tr>
<tr>
<td>Inflammation and Consequences</td>
<td>€520,719</td>
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<tr>
<td>Oncology</td>
<td>€506,111</td>
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<td>Research Groups</td>
<td>€700,964</td>
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<tr>
<td>Bioinformatics</td>
<td>€200,631</td>
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<tr>
<td>Clinical Neurosciences</td>
<td>€202,970</td>
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<tr>
<td>Cardiovascular Research</td>
<td>€203,384</td>
</tr>
<tr>
<td>Inflammation and Consequences</td>
<td>€93,579</td>
</tr>
</tbody>
</table>
In 2017, 46% of the overall expenses of the IZKF were spent on project funding, 14% on funding research groups and 32% on core facilities. A further 4% was spent on the administration office and 4% on the IZKF core laboratory.

In 2017, €2,271,997 was spent on project funding. The five research focus areas of the IZKF correspond to those of the Faculty of Medicine of the RWTH: Medicine and Technology, Cardiovascular Research, Clinical Neurosciences, Inflammation and Consequences, and Oncology.

€2,271,997 was spent on project funding. The five research focus areas of the IZKF correspond to those of the Faculty of Medicine of the RWTH: Medicine and Technology, Cardiovascular Research, Clinical Neurosciences, Inflammation and Consequences, and Oncology.

Core Facilities were funded with €1,601,790 in total. A new core facility was established in July 2017: the Flow Cytometry Facility. The Proteomics Facility was granted additional funding (€101,000) in order to purchase a new mass spectrometer in December 2017.
### Participating Institutes and Clinics

#### Clinical Departments

<table>
<thead>
<tr>
<th>Clinical Departments</th>
<th>2014-2017</th>
</tr>
</thead>
<tbody>
<tr>
<td>Department of Anaesthesiology</td>
<td>X</td>
</tr>
<tr>
<td>Internal Medicine I</td>
<td>X</td>
</tr>
<tr>
<td>Department of Cardiac and Thoracic Surgery</td>
<td>X</td>
</tr>
<tr>
<td>Department of Child and Adolescent Psychiatry</td>
<td>X</td>
</tr>
<tr>
<td>Department of Child and Adolescent Neuropsychology</td>
<td>X</td>
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<tr>
<td>Department of Dental Preservation</td>
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<tr>
<td>Department of Dermatology</td>
<td>X</td>
</tr>
<tr>
<td>Department of Diagn. and Interv. Radiology</td>
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</tr>
<tr>
<td>Department of Gynaecology and Obstetrics</td>
<td>X</td>
</tr>
<tr>
<td>Department of Gynaecological Endocrinology and Reproductive Medicine</td>
<td>X</td>
</tr>
<tr>
<td>Department of Neurosurgery</td>
<td>X</td>
</tr>
<tr>
<td>Department of Neurology</td>
<td>X</td>
</tr>
<tr>
<td>Department of Nuclear Medicine</td>
<td>X</td>
</tr>
<tr>
<td>Department of Ophthalmology</td>
<td>X</td>
</tr>
<tr>
<td>Department of Orthodontology</td>
<td>X</td>
</tr>
<tr>
<td>Department of Orthopaedics</td>
<td>X</td>
</tr>
<tr>
<td>Department of Otorhinolaryngology, Plastic Head, Neck Surgery</td>
<td>X</td>
</tr>
<tr>
<td>Department of Paediatric Medicine</td>
<td>X</td>
</tr>
<tr>
<td>Department of Paediatric Cardiology</td>
<td>X</td>
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<tr>
<td>Department of Palliative Medicine</td>
<td></td>
</tr>
<tr>
<td>Department of Phoniatrics, Pedaurology and Communication Disorders</td>
<td>X</td>
</tr>
<tr>
<td>Department of Plastic, Hand and Burns Surgery</td>
<td></td>
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<tr>
<td>Department of Prosthodontics and Dental Materials</td>
<td>X</td>
</tr>
<tr>
<td>Department of Psychiatry</td>
<td>X</td>
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<tr>
<td>Department of Research Area Dental Materials and Biomaterials Research</td>
<td>X</td>
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<tr>
<td>Department of Psychiatry</td>
<td>X</td>
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<tr>
<td>Department of Research Area Experimental Neuropsychiatry</td>
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</tbody>
</table>

#### Institutes

<table>
<thead>
<tr>
<th>Institutes</th>
<th>2014-2017</th>
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</thead>
<tbody>
<tr>
<td>Institute of Aerospace Medicine</td>
<td>X</td>
</tr>
<tr>
<td>Institute of Anatomy and Cell Biology</td>
<td>X</td>
</tr>
<tr>
<td>Institute of Biochemistry and Molecular Biology</td>
<td>X</td>
</tr>
<tr>
<td>Institute of Biochemistry and Molecular Cell Biology</td>
<td>X</td>
</tr>
<tr>
<td>Institute for Biochemistry and Molecular Immunology</td>
<td>X</td>
</tr>
<tr>
<td>Helmholtz-Institute for Biomedical Engineering (Applied Medical Engineering)</td>
<td>X</td>
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<tr>
<td>Helmholtz-Institute for Biomedical Engineering (Cardiovascular Engineering)</td>
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<tr>
<td>Helmholtz-Institute for Biomedical Engineering (Experimental Molecular Imaging)</td>
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<td>Helmholtz-Institute for Biomedical Engineering (Cell Biology)</td>
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<tr>
<td>Helmholtz-Institute for Biomedical Engineering (Biomaterials)</td>
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<tr>
<td>Institute of Clinical Chemistry and Pathobiotechnology</td>
<td>X</td>
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<tr>
<td>Institute of History, Theory and Ethics in Medicine</td>
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<tr>
<td>Institute of Human Genetics</td>
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<tr>
<td>Institute for Hygiene and Environmental Medicine</td>
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<tr>
<td>Institute of Immunology</td>
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<tr>
<td>Institute of Laboratory Animal Science</td>
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<tr>
<td>Institute of Medical Psychology and Medical Sociology</td>
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</table>
## 1.4 Participating Institutes and Clinics

<table>
<thead>
<tr>
<th>Institutes</th>
<th>2014-2017</th>
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<tbody>
<tr>
<td>Institute of Medical Informatics</td>
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<tr>
<td>Institute of Medical Statistics</td>
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<td>Institute of Medical Microbiology</td>
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<tr>
<td>Teaching and Research Area Virology</td>
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<tr>
<td>Institute of Molecular and Cellular Anatomy</td>
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<tr>
<td>Institute for Molecular Cardiovascular Research</td>
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<td>Institute of Neuromanatomy</td>
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<td>Institute of Neuropathology</td>
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<td>Institute of Pharmacology and Toxicology</td>
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<td>Teaching and Research Area Pharmacology</td>
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<td>Institute of Pathology</td>
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<td>Teaching and Research Area Tumour Pathology</td>
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<td>Institute of Physiology</td>
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<td>External Cooperation</td>
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<td>DWI – Leibniz Institute for Interactive Materials</td>
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<tr>
<td>AICES - Aachen Institute for Advanced Study in Computational Engineering Science</td>
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</table>

## 1.5 Output and Evaluation

The following table gives an overview of the achievements of the IZKF during the last ten years. Details about the different parameters, as well as titles of theses and publications can be found in the project report.

<table>
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<td>Articles</td>
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<td>Scientific degrees**</td>
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<tr>
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<td>Bachelor/Master</td>
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<tr>
<td>Qualification</td>
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</tbody>
</table>

* IZKF support must be mentioned in publications and presentations. Publications are relevant to the IZKF if the support is mentioned in the acknowledgement and/or in the affiliation.

The number of publications fluctuates year to year and depends on which stage the project is in during the funding period.

** Information about diploma theses and doctoral theses can be found in the project reports.
Established in 1995, the Aachen Interdisciplinary Center for Clinical Research (IZKF) is one of six nationwide ZKFs at universities (Aachen, Erlangen, Jena, Köln, Münster and Würzburg) today. All of the IZKFs emerged from the Federal Ministry of Education and Research’s initiative “Health Research 2000”. Every center developed its own research focus areas and structures. The task and goal of the IZKF Aachen is to strengthen the translational medical research stemming from its basic research and clinic. The IZKF at UKA considers itself as a development and strategy program of the RWTH Faculty of Medicine. By supporting first class research projects, it strives to greatly improve chances for fundraising high amounts of external funding.

The Mission of the IZKF Aachen
The mission of the IZKF Aachen is to improve the overall quality of the translational medical research, emanating from both fundamental and clinical research.

The main focus is placed on
- project funding
- funding of young researchers
- funding of core facilities.

The central aim is always the strategic further development of the Faculty of Medicine of RWTH Aachen.

Five Research Focus Areas
As stipulated by the constitution, the IZKF Aachen research focuses correspond to those of RWTH Aachen’s Faculty of Medicine:
- Medicine and Technology
- Cardiovascular Research
- Clinical Neurosciences
- Inflammation and Consequences
- Oncology

Joint Project Funding
A funding period in the IZKF Aachen stretches over three years. At the end of 2017 a three-year funding period ended. Depending on the available budget, projects with a shorter funding period can be granted during the actual funding period.

The IZKF Aachen exclusively funds research projects from researchers, who have already acquired external funding and have published material relevant to the project.

Start-up funding for the start of a research career can be applied for through the START-program.

All project submissions are reviewed by external experts, who take the research quality and feasibility into consideration. The number of approved projects is determined by the yearly budget of the IZKF of € 4.5 Mio.

Young Researcher Funding
The Aachen IZKF offers ambitious researchers with outstanding achievements an attractive possibility for funding through its researcher group. The researcher groups are assigned to a clinic or institute and can independently work there. The group heads are essentially or partly freed from their clinical responsibilities and can immediately devote themselves to research. Currently four groups are being funded.

Group leaders are equipped with first class research qualification, experience in acquiring external funding, and are chosen by the IZKF steering committee through a selection symposium. After a positive evaluation after an initial period of three years, the funding can be extended for another three years.

Core facilities:
Equipment and Expertise – Service for Research
In addition to project funding and young researcher funding, the IZKF also funds the equipment and carrying out of “Core Facilities”: central service units, in which equipment, expertise, and methods are available. The Core Facilities are available for all members of the Faculty of Medicine and are a valuable resource for an effective research environment.
| T11 | JOINT RESEARCH PROJECT Leube | p. 26 |
| Mechanobiology |
| T11-1 | Windoffer/Leube | p. 27 |
| Keratin-dependent mechanics of keratinocytes |
| T11-2 | W. Wagner | p. 29 |
| Cell-Biomaterial Interactions of iPSC-derived Mesenchymal Stromal Cells |
| T11-3 | Tohidnezhad/Jahr | p. 31 |
| Novel treatment options for tendinopathies |
| T11-4 | Fischer | p. 35 |
| 3DPrint - Mechanobiological effects of 3D-printed hydrogel-calcium phosphate composite materials on multipotent human mesenchymal stromal cells |
| T11-5 | Ludwig/Yildiz | p. 37 |
| Regulation of disintegrins and metalloproteinases ADAM by mechanical forces |

| T12 | JOINT RESEARCH PROJECT Cornelissen | p. 39 |
| EndOxy – Development of a biohybrid lung assist device |
| T12-1 | Cornelissen | p. 40 |
| Biochemical and biomechanical factors influencing the gas permeability of endothelial cells |
| T12-2 | Steinseifer/Arens | p. 42 |
| Development of an Oxygenator Suitable for an Endothelialization |
| T12-3 | Jockenhövel | p. 44 |
| Textile membrane |
| T12-4 | Wessling | p. 46 |
| 3-dimensional membranes for biomimetic oxygenators |
Mechanobiology

Leube, R. (Institute of Molecular and Cellular Anatomy)

The overarching research question of the mechanobiology consortium is to find out how mechanical forces control and shape cells and tissues. We therefore wanted (i) to develop and apply devices for controlled application of defined mechanical forces on cultured cells from outside and within and (ii) to investigate how mechanical forces drive physiologically and clinically relevant cellular behaviour. To achieve these goals we tightly cooperated with research groups at RWTH Aachen, DWI and FZJ. The innovative devices, methods and materials included multipolar magnetic tweezers (SP1), composite hydrogels and elastomers with defined stiffness (SP2, SP4), dynamic cell stretchers (SP3), 3D bioprinting (SP4), and combination of endothelial flow culture with real time imaging (SP5). Major insights gained concern the contribution of the keratin-desmosome scaffold to epithelial tissue mechanics (SP1), of substrate stiffness to stem cell differentiation (SP2, SP4), of dynamic stretching to tenocyte differentiation (SP3), and of endothelial shear stress to metalloproteinase expression and endothelial cell survival (SP5). In sum, we made progress toward a mechanical understanding of tissue differentiation and function. The progress was communicated in more than 20 peer-reviewed publications. We were able to obtain substantial extramural funding (>7x10^6 €) from the EU, DFG, BMBF and other sources. Intense networking was the basis for a successful draft proposal for a DFG-funded research training grant.

Keratin-dependent mechanics of keratinocytes

Windoffer, R. (Institute of Molecular and Cellular Anatomy)

The aim of this project was the investigation of the still poorly understood importance of keratin intermediate filaments for the mechanical properties of epithelial cells. The project was performed in close collaboration with U. Schnakenberg (IWE1, RWTH Aachen University) and R. Merkel (ICS-7, FZJ) yielding the following results:
- Downregulation of the cytolinker plectin, which has binding sites for actin and intermediate filaments, showed no detectable effect on the intracellular force balance and had only minor effects on keratin network organization and dynamics (Moch et al. 2016).
- Using superresolution microscopy in different epithelial cell lines we uncovered the architecture of a rim and spoke system of keratin filaments connected to cell contacts. We posit that this system fulfills a major biomechanical function together with the cortical actin cytoskeleton (Quinlan et al. 2017).
- To investigate the influence of keratin phosphorylation on cell mechanics (Sawant and Leube 2017) we expressed phosphomimetic keratin mutants in different backgrounds. We showed that a single phosphomimetic mutation affects cell stiffness and viscoelasticity and also alters cell proliferation and viability (Sawant et al. 2017; unpublished observations)
- To validate the observed biomechanical and dynamic properties of the keratin cytoskeleton in cell culture we analyzed keratin network architecture and dynamics in living murine embryos (Schwarz et al. 2015).
- Reviews on the intermediate filament motility (Leube et al. 2017), the impact of intermediate filaments on mitochondrial structure and function (Schwarz and Leube 2016), and the regulation of focal adhesions by intermediate filaments (Leube et al. 2015) were published.
- We started mathematical modelling of keratin dynamics (Portet et al. 2015).

By reaching the major objectives of the project we were able to obtain substantial third party funding and are now in a good position for further applications focusing specifically on the mechanobiology of keratin intermediate filaments in epithelial tissues.
Publications


Cell-Biomaterial Interactions of iPSC-derived Mesenchymal Stromal Cells
Wagner, W. (Institute for Biomedical Engineering – Stem Cell Biology and Cellular Engineering)

Induced pluripotent stem cells (iPSCs) can be differentiated toward mesenchymal stromal cells (MSCs) – but this transition remains incomplete, at least on epigenetic level. It has been suggested that matrix elasticity directs cell fate decisions. Therefore, we followed the hypothesis that differentiation of iPSCs toward MSCs (iMSCs) can be enhanced on very soft hydrogels (<30 Pa) made of human platelet lysate (hPL).

We established five different culture conditions and initially compared culture expansion of primary MSCs: 1) As a reference, we used conventional TCP covered with culture medium that contained 10% hPL (hPL-medium). 2) As an alternative to hPL-medium, we established hPL-plasma, which lacks coagulation factors and therefore makes use of heparin dispensable. 3) Furthermore, hPL-gel was used as a substrate for MSC culture. 4) We added thrombin (TN) to hPL-gel to accelerate gel formation. 5) MSCs were trapped inside hPL-gel with addition of TN. Subsequently, iPSCs were differentiated toward MSCs in the above mentioned culture conditions (Figure 1A). However, when embedded in hPL-gel, iPSCs did not differentiate toward MSCs.

We demonstrate that hPL-gel supports growth of primary MSCs, even in 3D, with pronounced deposition of extracellular matrix, although it hardly influences gene expression profiles or in vitro differentiation of MSCs. Furthermore, we could effectively differentiate iPSCs toward MSCs on hPL-gel. Unexpectedly, this complex differentiation process is not affected by the soft substrate: iMSCs generated on tissue culture plastic (TCP) or hPL-gel have the same morphology, immunophenotype, differentiation potential, and gene expression profiles. Furthermore, global DNA methylation patterns are essentially identical in iMSCs generated on TCP or hPL-gel, indicating that they are epigenetically alike. Taken together, hPL-gel provides a powerful matrix to support growth, ECM deposition, and differentiation of MSCs and iMSCs – however, this soft hydrogel does not impact on lineage-specific differentiation.

Funding period: 01.07.2014 – 31.12.2017
Staff: TV-L13 (65%): Goetzke, R.

Applied third-party funding (DFG, BMBF, EU, foundations)
Leube, R. Consequences of desmosome anchorage for keratin network dynamics and organization DFG LE566/22-1 (SPP 1782): 2015-2018 € 186,500
Leube, R./Windoffer, R. The keratin filament cycle of assembly and disassembly DFG LE566/18-2 and WI1731/8-2: 2017-2020 € 452,400
Leube, R. (coordinator)/ Windoffer, R. Integrated component cycling in epithelial cell motility HORIZON 2020 KIS020 MSCA-ITN InCeM 642866: 2015-2018 € 3,884,000, SP: € 420,000

Promoting of young researchers
Doctoral Theses
Al Beisti, M. 2017 RWTH Aachen, Faculty 1 Keratin-dependent mechanics of keratinocytes

Publications

Figure 1: Comparison of culture conditions for MSCs and iPSC-derived MSCs.

A) Schematic presentation of culture on tissue culture plastic (TCP) either with hPL-medium or hPL-serum (hPL-medium after removal of coagulation factors); on hPL-gel either without (w/o) or with thrombin (TN); and embedded in hPL-gel. B) Phase contrast images of MSCs cultured in the different culture conditions. C,D) Unpaired limma-t-test (adjusted p-value < 0.05) demonstrated marked differences in gene expression and DNA methylation between MSCs and iMSCs, whereas culture and differentiation on stiff TCP or hydrogel did hardly have significant impact on gene expression or DNA methylation profiles.

Applied third-party funding (DFG, BMBF, EU, foundations)

Wagner, W./ Wessling, M. Hollow Fiber Membrane Reactor for Cultivation of 3D Stem Cell Tissues ERS Seed Fund 01/2018-01/2019 € 65,000

Wagner, W./ Gillner, A. The Impact of sub-µm Surface Topography on Pluripotent Stem Cells DFG 3 years requested submitted; pending result

Promoting of young researchers

Doctoral Theses

Goetzke, R. Ongoing RWTH Aachen, Faculty T1/10 Cell-Biomaterial Interactions of iPSC-derived Mesenchymal Stromal Cells

Novel treatment options for tendinopathies

Tohidnezhad, M. (Department of Anatomy and Cell Biology)
Jahr, H. (Department of Orthopaedic Surgery)

Cues from mechanical loading seem to be essential for maintaining the phenotypic stability of tenocytes and tendons. The aim of this study was to develop and use novel mechano-reactors in order to improve regenerative properties of this tissue. Major achievements of this project comprise i) the development of different types of novel mechano-bioreactors for dedicated applications, comprising mechano-bioreactors specifically designed to study the responses of i) isolated, in vitro cultured, tendon cells (i.e., tenocytes) and iii) to study their responses in situ in whole tendon ex vivo stretching reactors. We further identified rather catabolic and mainly anabolic stretching regimes and characterized cellular responses in novel biomimetic tendon repair scaffolds.

Based on their overall performance, two bioreactor types were further studied in more detail and are briefly described below. For in vitro experiments, isolated primary murine tenocytes were either seeded into biomimetic tendon repair scaffolds (figure 1 d) or stretched in a novel multi-parallel throughout mechano-reactor that was developed with project partner FZ Jülich. This so-called Cell Stretcher X6 (figure 1 a). This open platform-type of mechano-reactor is well suited for short- to medium-term experiments. Currently, modifications are ongoing to improve the design further and make it applicable for long-term studies. Our proof-of-concept studies comprised RNA isolation and RT-qPCR analyses as well as immunohistochemical (IHC) staining. The former was used to profile expression of tendon-specific markers or matrix turnover indicators based on their mRNA abundances in a semi-throughput approach, while the latter was used to monitor expression of effector molecules (i.e., marker proteins) in real-time.

While studying responses of isolated cells may be scientifically relevant under certain conditions, studying the response to loading of intact tissues may be more relevant to answer other questions. Therefore, we further aimed at developing ex vivo reactors. In collaboration with the Institut für allgemeine Mechanik (IAM), we co-developed a whole tendon tissue mechano-reactor. We focused on studying tendons of the musculus flexor digitorum longus from rats: isolated tissue was subjected to monoaxial stretching in a custom-made bioreactor (figure 1 c) for 6 h, 24 h or 48 h at 1 Hz. To investigate the effect of stretching on the elastic strain of the tendon, the real time reaction forces were obtained. Molecular markers were used to monitor phenotypic stability of tenocytes in monolayer cultures, 3D cultures or during ex vivo tissue cultivation. After stimulation, marker expression was quantified by IHC staining and real-time PCR. A stretch frequency of 1 Hz using the Cell Stretcher X6 increased expression of e.g. tenomodulin (TNMD) and activity of scleraxis (SCX) in tenocytes. Stretch frequencies of 2 Hz upregulated matrix-metalloproteases-1 (MMP-1) and -13 in tenocytes (figure 1 b). The stiffness data of the tendons could be recorded online and real-time during the cultivation of the tissue.
Figure 1: Two selected examples of newly developed mechano-bioreactors. Cell Stretcher X6 (a), developed in collaboration with FZ Jülich, is specifically designed to mechano-biologically stimulate primary (here: murine) tenocytes in vitro after seeding them into bio-mimetic tendon repair scaffolds (b). Expression of relevant markers, like matrix remodeling proteases (e.g., MMP-1) or tendon-specific marker tenomodulin (TNMD) were analyzed by IHC (b). For ex vivo mechano-stimulation of intact tendon tissues another type of bioreactor (c) was developed in collaboration with the Institut für allgemeine Mechanik (IAM). In this device, isolated tendons of the musculus flexor digitorum longus from rats were subjected to longitudinal cyclic monoaxial stretching at e.g. 1 Hz (e). Generic matrix remodeling potential was assessed by measuring expression of important matrix metalloproteinases (e.g., MMP-13) by RT-qPCR, while the activity of selected tenocyte-specific transcription factors, like e.g. scleraxis (Scx), a member of the basic helix-loop-helix (bHLH) superfamily of transcription factors, was evaluated based on relative differences in nuclear translocation under influence of different stimulation regimes (f). **, ****; p<0.05, <0.001, respectively.

With these parameters, nuclear translocation (i.e., activity) of tendon-specific marker SCX increased under stretch as compared to the non-stretched control, as did the stiffness of the tendon.

However, this "training" effect was temporary as tendon stiffness values dropped to baseline levels within 24h (figure 1 e). The increased MMP-1 expression and the reduced SCX activity in the second half of the treatment regime confirmed this impression (figure 1 f). Nevertheless, our approach enabled the determination of activation pattern of potentially quicky mechano-responsive key regulators of cellular phenotype, like e.g. transcription factors, by, among others, real time-suitable screening of their nuclear localization kinetics (figure 1 b). We were further able to study the impact of the stretching frequency (e.g., 1 Hz vs. 2 Hz) longitudinally and, among others, revealed a prominent change in its expression over time (figure 1 b).

In summary, in this project cultivation of tenocytes and tendon tissue in the novel bioreactors was accomplished. Comparison of different bioreactor designs and settings showed that a well-chosen frequency during the cell- and tissue- stretching regime appears to be beneficial for the tenocyte phenotype, based on the selected marker gene expression. The Cell Stretcher X6 appeared to be suitable to improve current in vitro cultivation protocols for tenocytes and a stretching frequency of 1 Hz is recommended to optimally stabilize the tenocyte phenotype and improve tenocyte physiology in vitro. Using molecular biological analysis and online mechanical analysis of the tissue stiffness, it is now possible to improve the cultivation condition of these challenging tissues for future tissue engineering applications.

Publications

Awards
Jahr, H. - Familie Klee Innovation Award, 2017
The aim of the project was the synthesis of nano-calcium phosphate (CaP) particles and the investigation of cellular response of human mesenchymal stromal cells (hMSCs) embedded in hydrogel with nano-calcium-phosphate composite materials along with investigation of the printability and cell viability of the cell-laden hydrogel-calcium-phosphate composites using customized 3D bioprinter. The inspiration to use nanostructured biomaterials in bone regeneration comes from the native bone architecture. Several studies have shown that nano-CaP biomaterials exhibit good physicochemical and biological characteristics due to being more similar to bone nanocrystals. Microwave assisted method was utilized in synthesizing nano-calcium phosphate particles because it is relatively quick and there is direct transmission of energy to the entire volume and avoiding temperature gradients which plays role in improvement in size, purity, and size distribution. The synthesized nano particles were pure hydroxyapatite (HAp) with size ranging from 60–500 nm. These particles were incorporated into different hydrogels (agarose and agarose/collagen blends) at concentrations of 0.5 mg/ml and 5 mg/ml. The stiffness and viscosity of the cross-linked composites was measured by compression testing and using a rheometer, respectively. The cellular response of hMSCs cells embedded in the 3D composite materials with varying elasticity was studied with respect to morphology, cell viability, and osteogenesis. Human MSCs showed very good cell viability, cell spreading, and differentiation capacities in less stiff composites with 0.5 mg/ml nano-HAp. The printability of the composite materials was investigated using customized 3D bioprinter which prints drop-on-demand and layer-by-layer techniques. Valves of 150 μm, 300 μm and 600 μm size were used. Most of agarose/collagen with calcium phosphate blends could not be printed using 150 and 600 μm valves. However, all composite blends showed good printability using the 300 μm valve. The cell viability of human mesenchymal stromal cells embedded in these composite materials were high even after printing. Figure 1 shows a 3D-printed construct of agarose with nano-HAp composite material.
Figure 1: Novel agarose-calcium-phosphate composite construct achieved by drop-on-demand 3D bioprinting technique.

**Applied third-party funding (DFG, BMBF, EU, foundations)**

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**Promoting of young researchers**

**Doctoral Theses**

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<td>Sen, K.</td>
<td>Ongoing</td>
<td>RWTH Aachen, Faculty 5</td>
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<td>Mechanobiological effects of 3D-printed hydrogel-calcium-phosphate composites on human mesenchymal stromal cells</td>
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**Regulation of disintegrin and etalloproteinases (ADAM) by mechanical forces**

Yildiz, D. (Institute for Pharmacology and Toxicology)
Ludwig, A. (Institute for Pharmacology and Toxicology)

Flow conditions critically regulate endothelial cell survival in the vasculature. Reduced shear stress resulting from disturbed blood flow can impair endothelial integrity and drive the development of vascular inflammatory lesions. In the previous years of this funding period we found that proteases of the ADAM family are regulated under flow and inflammatory conditions. We now studied the regulation of important adapter molecules for ADAM proteases. Transcriptomic analysis and quantitative RT-PCR revealed that the rhomboid pseudoprotease iRHOM2 is upregulated under inflammatory conditions whereas iROHM1 is upregulated under flow conditions (Figure 1A,B). The latter induction is mediated by the transcription factor KLF2 which is also upregulated in response to shear stress. KLF2 can be induced in endothelial cells by treatment with simvastatin and this is associated with an induction of iRHOM1. Both effects are suppressed by counteracting simvastatin with geranylgeranylpyrophosphate (GGPP) (Figure 1C). Overexpression of KLF2 promotes iRHOM1 expression and siRNA knockdown of KLF2 prevents the induction of iRHOM1 in response to shear stress. Functionally, iRHOM2 and to some degree also iRHOM1 have been implicated in maturation and activity regulation of ADAM17. In fact, induction of iRHOM2 by inflammatory stimuli is associated with enhanced maturation and surface expression of ADAM17 (Figure 1D,E). These effects can be prevented by siRNA mediated knockdown of iROHM2. Interestingly, induction of iROHM1 by flow conditions is also associated with increased surface expression of ADAM17 without changing ADAM17 maturation. siRNA mediated silencing of iRHOM1 or 2 revealed that both iRHOMs are required to maintain mRNA expression of several ADAM proteases including ADAM17 (Figure 1F). We propose that regulation of ADAM17 on endothelial cells can occur on different levels: directly on the protein level resulting from upregulation and binding of iRHOM2 under inflammatory conditions and indirectly on the transcriptional level which may involve upregulation of iRHOM1 under flow conditions.

**Publications**


**Applied third-party funding (DFG, BMBF, EU, foundations)**

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**Promoting of young researchers**

**Bachelor/Master Theses**

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<td>Regulation of ADAM1 proteases by TIMPs</td>
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Funding period: 01.07.2014 - 31.12.2017

Staff: TV-L13 (60%): Wozniak, J.
**EndOxy – Development of a biohybrid lung assist device**

Patients suffering from severe chronic obstructive lung disease (COPD) develop chronic hypercapnic respiratory failure. These patients experience a markedly reduced quality of life, suffering from constant dyspnea. Current long-term mechanical pulmonary ventilation will relieve this situation for some, but not all patients.

Long-term application of Extracorporeal CO2 Removal might extend therapeutic options in end-stage COPD but the development of an intracorporeal system has been hampered by the issues of anticoagulation and limited hemocompatibility. Coating the oxygenator’s surface with endothelial cells might be the first step towards solving these issues.

To this end, new, 3-dimensionally structured gas permeable membranes were developed and tested. Also, a new oxygenator design that takes into account the necessities of a cell coating was created and a test system was built. The effects of cell coating on gas transfer properties and strategies for protecting cells from high oxygen tension were investigated.

In cooperation with partners from the i3tm initiative of the RWTH as well as external partners working on this topic, the DFG priority program “Towards an implantable lung” was set up to continue the developments initiated by the IZKF ENDOXY consortium.

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**Doctoral Theses**

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**Appointments to other universities**

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<td>Homburg</td>
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**Figure 1:** Regulation of ADAM17, iRHOM1 and iRHOM2 on endothelial cells in response to flow conditions and inflammatory stimulation: A-B) Endothelial cells were cultured under flow or static conditions and additionally stimulated with TNF. Subsequently, iRHOM1 and 2 mRNA expression was studied. B) Endothelial cells were treated with simvastatin with and without GGPP and investigated for iRHOM1 mRNA expression. C-E) Endothelial cells were cultured under flow or static conditions, additionally stimulated with TNF and subsequently studied for ADAM17 mRNA and surface expression. F) Effect of siRNA mediated iRHOM1 or 2 knock down on ADAM17 mRNA expression. Data are shown as mean and SD of at least 3 experiments (A-D,F) or as representative histograms (E). Statistically significant differences (p<0.05) are indicated.
Biochemical and biomechanical factors influencing the gas permeability of endothelial cells

Cornelissen, C. (Clinic for Cardiology, Pneumology, Angiology and Internal Intensive Medicine (Medical Clinic I) & Helmholtz Institut, Applied Medical Engineering – Tissue Engineering & Textile Implants)

RGD-coated silicone membranes proved suitable for long-term dynamic cultivation of endothelial cells. We were able to maintain an integral cell layer for more than four weeks (s. Figure 1) and thus attained a cultivation period that is comparable to the certified long-term use of today’s oxygenators. In vitro blood trials have further demonstrated the stability of the endothelial cell layer exposed to blood flow. Initial gas transfer tests according to ISO 7199 have been successfully conducted in a model system of a biohybrid lung. While a significant impact of the endothelial cell layer on gas transfer performance has not been observed so far, more extensive testing is required to evaluate the impact of long-term dynamic cultivation on gas transfer performance. Within the scope of the EndOxy project various membrane systems have been evaluated regarding endothelialisation, gas transfer performance and long-term dynamic cultivation. RGD-coated silicone identified as the most suitable one to meet the requirements of a biohybrid lung assist device. Together with the very promising results from the in vitro blood testing we were able to demonstrate the feasibility of a biohybrid lung.

Figure 1: Endothelial cell layer after 28 days of dynamic cultivation with a wall shear stress of 4 dyne/cm². Endothelial cells are positive for typical endothelial cell markers as has been shown by fluorescence staining against CD 31 (red) and von Willebrand factor (green). Cell nuclei were counterstained with DAPI (blue).
Development of an Oxygenator Suitable for an Endothelialization

Steinsiefer, U.
Arens, J.
(Helmholtz Institut für Biomedizinische Technik, Institut für Applied Medical Engineering – L&F Tissue Engineering & Textile Implants)

We developed a machined laboratory sample of an endothelializable oxygenator for in-vitro testing. Based on the results from flow simulations of the bioreactor system proven feasible for the cultivation of the gas exchange membranes the fluid mechanical characteristics were directly transferred to the laboratory sample designed as a flat sheet membrane oxygenator. The technical design allows a process chain incorporating all steps for the supply of a biohybrid lung such as pre-assembling, biofunctionalization, sterilization, assembly and endothelialization.

An alternative concept was under investigation incorporating intraluminal flow membranes. The concept suggests a more compact design with similar flow conditions. Preliminary gas transfer tests have been performed with a small-sized laboratory sample to obtain a proof of principle. An up-scaled version with the focus on reduction of the fabrication effort has been designed.

Numerical simulations have been performed to evaluate different shaped 3D-Membranes as potential blood interface for more hemocompatible and efficient artificial lung. In comparison to current state of the art hollow fiber arrangements the numerical optimization reveals different designs with an increase of gas transfer efficiency and reduced loss of hemodynamical energy.

Further, expert interviews with regarding technical, regulatory and biological requirements have been performed to explore the potential of a biohybrid artificial lung and the focus of possible future research projects within the scope of endothelializable artificial lungs.

Applied third-party funding (DFG, BMBF, EU, foundations)

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Promoting of young researchers

**Bachelor/Master Theses**

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**Doctoral Theses**

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Textile membrane

Jockenhövel, S. (NRW-Schwerpunktprofessur Biohybrid & Medical Textiles (BioTex) at Institute for Textile Engineering)

The extension of the project in 2017 was used to evaluate a novel sandwich production of electrospun membranes and coatings. Therefore two materials were investigated. The first one was sulfonated polyetheretherketone (sPEEK) which was synthesized, the process parameters were determined and then sPEEK spun into flat membranes for characterization. Different combinations were characterized the e-spun sPEEK membrane itself as well as a sPEEK coated silicone membrane. Unfortunately in the first evaluation the results showed a cytotoxicity of the e-spun sPEEK membrane although the material itself is used in biomedical applications so that the production process could be the reason for the increased cytotoxicity. Therefore the material was replaced by polyvinylidene fluoride (PVDF). PVDF is a commonly used polymer in medical applications it is non-degradable and exhibits low immune reaction as well as a high biocompatibility. The spinning parameters for PVDF were established and flat silicone membranes were coated with PVDF membranes for better cell attachment. The produced flat oxygenation membranes were evaluated regarding their mechanical strength, watertightness and burst strength. Further research which will be conducted as a follow up to this project will include the testing if an integration of the composite membranes from silicone and e-spun PVDF into an oxygenator prototype will be possible. Furthermore the cell material interaction will be investigated via XTT-assays and a direct seeding of cells onto the membranes to show the cyto- and biocompatibility in-vitro of the developed membranes. The current technology is not only interesting for oxygenator membranes but also as cell carrier for other application in regenerative medicine.

Publications

Applied third-party funding (DFG, BMBF, EU, foundations)

<table>
<thead>
<tr>
<th>Project Description</th>
<th>Funding Agency</th>
<th>Status</th>
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<tbody>
<tr>
<td>Jockenhövel, S./Tenbrock, K.</td>
<td>Endothelial Cell Lining of Oxygenator Membranes (EndOxy) - Influence on inflammatory pathways and immune-endothelial cell interaction.</td>
<td>DFG</td>
</tr>
<tr>
<td>Jockenhövel, S./Cornelissen, C./Arens, J.</td>
<td>EndOxy - Development of an endothelialized oxygenator for enhanced - biocompatibility and gas exchange performance</td>
<td>DFG</td>
</tr>
<tr>
<td>Wessling, M./Arens, J./Cornelissen, C.</td>
<td>3D printed membrane architectures for ECMO application</td>
<td>DFG</td>
</tr>
</tbody>
</table>

Promoting of young researchers

Bachelor/Master Theses
Engesser, F. | Ongoing | RWTH Aachen, Faculty 4 | Development of an e-spun PVDF coating for silicone based oxygenator membranes |

Doctoral Theses
Krusse, M. | Ongoing | RWTH Aachen, Faculty 4 | e-spun technologies in regenerative medicine |
3-dimensional membranes for biomimetic oxygenators

Wessling, M. (DWI Leibniz Institute for Interactive Materials RWTH Aachen)

Extracorporeal membrane oxygenators (ECMOs) are membrane based artificial lung therapies, which are used i.e. to support patients with acute respiratory failure where blood is oxygenated and carbon dioxide is removed. Hollow fiber based systems are conventionally used for ECMO application but are limited in intervening the blood flow design since blood only allows a small window of operating extracorporeal (e.g. sensitive towards shear stress). With additive manufacturing, we can tailor the flow profile and surface to volume ratio for achieving physiological flow profiles, postpone thrombosis and hemolysis as well as gas exchange efficiency in order to evaluate a membrane design for blood oxygenation. So far, several 3D TPMS (triplly periodic minimal surface) geometries were fabricated out of pure PDMS (Polydimethylsiloxane), a highly gas permeable membrane material. However, with the present technique only thick membranes (mm scale) can be achieved which increase the transport resistance for gases and hence are not applicable for oxygenators. To overcome this, a novel technology was established to achieve high flux composite membranes in which a porous support with PVDF (Polyvinylidene fluoride) and on top of that a dense layer with PDMS was fabricated (see fig. 1A).

In parallel to this, PDMS membranes were post functionalized via two different functionalization methods to enable covalent binding sides for endothelial cells: in one approach, an interfacial polymerization technique was applied but stable films were not able to obtain so far. In another approach, functional polymer brushes with alternating antifouling and peptide binding sides were successfully bound to PDMS. A covalent cell layer was cultured on these surfaces and the cells were even capable of withstanding an external flow.

Figure 1: 3D printed PVDF membrane (A) with highly porous structure (B). Endothelial cells cultured on PDMS flat sheet membranes under static (C) and dynamic conditions (D, E). Staining: nucleus with Dapi (blue), cytoskeleton with Alexa Fluor 488 (green), focal adhesion points with vinculin (red).

Funding period: 01.07.2014 - 31.12.2017

Staff:
TV-L13 (50%): Djeljadini, S.

Promoting of young researchers
Bachelor/Master Theses

- Lazar, N. MA Thesis Ongoing RWTH Aachen, Faculty 4 Bio functionalization of 3D printed PDMS membranes for enhanced endothelialisation under shear stress
- Quatzmann, E. MA Thesis Ongoing Fh Jülich Investigation of gas transfer through an artificial endothelial cell barrier under shear stress
- Röders, M. MA Thesis Ongoing RWTH Aachen, Faculty 1 Endothelialisation of PDMS membrane functionalized with polymer brushes for long term application of extracorporeal membrane oxygenators
- Hartmann, N. MA Thesis 2017 RWTH Aachen, Faculty 4 Additive Manufacturing of 3D Thin Film Composite Membranes for Extracorporeal Blood Oxygenation
- Rose, I. MA Thesis 2017 RWTH Aachen, Faculty 4 Dynamic cell cultivation on post functionalized 3D printed PDMS membranes for biohybrid oxygenators

Doctoral Theses

- Djeljadini, S. Ongoing RWTH Aachen, Faculty 4 Membrane based dynamic tissue engineering
K7 | JOINT RESEARCH PROJECT

Marx/Jahnen-Dechent p. 50
Cardiovascular disease in the vulnerable patient

K7-1 | Noels p. 52
Role of CXCR4 ligands and their regulators in platelets: implications for the vulnerable patient

K7-2 | Schütt/Schlieper p. 55
Chronic kidney disease and coagulation: the impact of clot structure

K7-3 | Floege/Boor p. 57
The role of PDGFs in uremic cardiomyopathy

K7-4 | Krusche/Leube p. 59
The vulnerable myocardium of desmoglein 2-mutant mice

K7-5 | Jahnen-Dechent p. 62
Role of the plasma protein fetuin-A in lipidic calcified debris metabolism

K7-6 | van Zandvoort/Kiessling p. 65
From cells to animals and back: an integrated optical imaging platform for cardiovascular research

K7-7 | Marx/Lebherz p. 67
Influence of C-peptide on cardiac metabolism and function in ischemia/reperfusion injury

K7-8 | Stoppe/Goetzenich p. 68
Novel exacerbating factors in myocardial ischemia/reperfusion

PROJECTS

Cardiovascular Research
Cardiovascular disease in the vulnerable patient

Marx, N. (Department of Internal Medicine I)
Jahnen-Dechent, W. (Helmholtz Institute for Biomedical Research, Biointerface Laboratory)

The joint project “Cardiovascular disease in the vulnerable patient” is examining critical pathophysiological processes of the vulnerable patient with chronic kidney disease (CKD) and/or diabetes and is aiming to gain new insight into the interaction of the three determinants of cardiovascular risk - vulnerable vessel, vulnerable blood and vulnerable myocardium - in these high risk patients.

The current joint proposal was initiated to earn large external collaborative grants improving the visibility of the Medical Faculty of RWTH Aachen University. Accordingly members of the IZKF consortium were instrumental in the TR SFB grant application shared between University of Homburg and RWTH Aachen University, entitled "Mechanisms of cardiovascular complications in accelerated chronic kidney disease". This application was positively evaluated during a site visit last September and finally approved by the DFG senate in November. The project has duly started in January 2018. In addition, 4 PIs / co PIs also supervise PhD students in the EU funded Marie-Curie Innovative Training Network (ITN) – INTRICARE (International Network for Training on Risks of vascular Intimal Calcification And roads) project.

Overall approved external funding based on the work of the consortium was 7.4 Mio € in 2015-2017. Research will be published in due course naming IZKF Aachen as a funding source.

Funding period: 01.07.2014 - 31.12.2017
Total Budget 2017: € 409,104

Figure 1: Projects from the original IZKF consortium “Cardiovascular disease in the vulnerable patient” that are involved in the approved SFB-TR 2019 “Mechanisms of cardiovascular complications in accelerated chronic kidney disease”
Role of CXCR4 ligands and their regulators in platelets: implications for the vulnerable patient

Noels, H. (Institute for Molecular Cardiovascular Research)

Platelets are subjected to multiple inhibiting and activating substances. Dipeptidyl peptidases (DPP) carry out a multitude of physiological functions. DPP4 regulates the CXCR4 ligand CXCL12 and glucagon-like peptide 1 (GLP1), among other substrates, and DPP4 inhibitors are being used for the treatment of patients with type 2 diabetes mellitus, a patient group with high thrombotic and cardiovascular risk. Our results indicate that plasma-derived DPP4 has a positive priming role in platelet activation. If DPP4 activity is blocked or deficient, the active form of GLP1 accumulates in plasma, resulting in reduced positive platelet priming through the GLP1 receptor and, hence, lower platelet activation via cAMP elevation (Figure 1).

In addition to diabetes, also chronic kidney disease (CKD) highly increases the risk of cardiovascular disease (CVD), caused by the accumulation of hydrophobic uremic toxins in the blood of these patients due to a failing kidney function. Although CKD patients in the last stage of disease are treated with dialysis to replace the kidney function, the removal of hydrophobic uremic toxins from the blood by current dialysis techniques is difficult due to the high binding affinity of these toxins to plasma proteins. As an extension of our project, we developed and investigated an extracorporeal adsorber particle with a modified surface for the adsorption of hydrophobic uremic toxins to improve the existing high-flux hemodialysis techniques. The “Netphob” particle shows a high adsorption capacity to all tested hydrophobic uremic toxins and a good hemocompatibility, also in relation to platelets. Thus, the newly synthesized “Netphob” particle is an interesting candidate for further in vivo studies with the aim to improve the treatment of vulnerable CKD patients.

Further, platelets play a crucial role in the development of atherosclerosis, which underlies a majority of cardiac events. We could show that CSN5 suppresses macrophage inflammation and that mice lacking myeloid CSN5 develop larger atherosclerotic lesions. The NEDDylation inhibitor MLN492, mimicking CSN5 overexpression, attenuates early atherosclerosis. With MLN4924 in clinical trials, deNEDDylation approaches may qualify as therapeutics to reduce early-stage atherogenesis (Asare et al, PNAS 2017).

Publications

Applied third-party funding (DFG, BMBF, EU, foundations)
Noels, H.,Altm, N. Inflammatory mediators linking CKD to adverse cardiac remodeling after myocardial infarction DFG - SFB/TRR219-Projekt M-05 01/2018-12/2021 € 401,160
Noels, H. Marie-Curie ITN CaReSyAn EU H2020-ESR11 01/2018-12/2021 € 747,649
A compact fibrin clot structure and impaired fibrinolysis are associated with more severe cardiovascular disease. Both, subjects with diabetes and chronic kidney disease (CKD), exhibit a prothrombotic clot structure characterized by small pores and resistance to fibrinolysis. The aim of the present project was to investigate whether complement activation products contribute to altered fibrinolysis in patients with diabetes and/or CKD and whether experimental uremia alters microembolus extravasation in mice. In 2017 we published a paper about clot structure as a potent mortality risk factor in hemodialysis patients in JASN. We investigated fibrin clot structure parameters and the impact on mortality in a prospective cohort of 171 chronic hemodialysis patients (59±11y, 54% male) using a validated turbidimetric assay. Kaplan Meier analysis revealed that hemodialysis patients with a denser clot structure had an increased all-cause and cardiovascular mortality risk (log-rank p=0.004 and p=0.003, respectively). Multivariate Cox regression models (adjusted for age, diabetes, gender, duration of dialysis, or adjusted for fibrinogen, CRP and complement C3) confirmed that denser clots are independently related to mortality risk. As complement C3 was not associated with mortality in CKD we focused on alterations of fibrinogen and performed further analysis investigating posttranslational modifications. While purified fibrinogen from healthy controls displayed no posttranslational modifications, fibrinogen from hemodialysis patients was glycosylated and guanidinylated. Clots made of purified fibrinogen from hemodialysis patients exhibited significantly thinner fibers compared to control individuals (63±1.9 nm and 77±2.2 nm respectively; p<0.001) as assessed by electron microscopy (Figure 1). This might at least partially be due to posttranslational modifications as in vitro guanidinylation of fibrinogen from healthy subjects resulted in an increased formation of thinner fibers. Thus, in HD patients a denser clot structure is a potent independent risk factor for mortality. As for our second goal, we had problems to visualize extravasation of microemboli using our standard mouse models. We further started to investigate the impact of complement activation on clot properties. First results in healthy individuals demonstrated a prothrombotic clot structure with thinner fibrin fibres and longer fibrinolysis. The role of single complement components and the impact of CKD will be investigated in more detail in the upcoming SFB/TRR219 project C-07.

Publications

Funding period: 01.07.2014 - 31.12.2017
Staff:
TV-L13 (50%): Maxeiner, S.
The role of PDGFs in uremic cardiomyopathy

Floegge, J. (Department of Internal Medicine II)
Boor, P. (Department of Pathology and Internal Medicine II)

Myocardial changes in chronic kidney disease (CKD) are considered a major contributor to the excessive cardiovascular morbidity of CKD patients, including their most frequent cardiac cause of death, sudden cardiac death. In this project, we have characterized various mouse models of progressive kidney injury/renal fibrosis with respect to functional and morphological cardiac alterations, in particular the Col4a3 deficient mice, i.e. the model of Alport syndrome and a progressive glomerulopathy, the remnant kidney model (also known as 5/6 nephrectomy), which induces glomerular hypertrophy and glomerulosclerosis, and the adenine model, a model of progressive crystal induced renal damage resulting in prominent uremia. We found that CKD mice exhibited arrhythmias and had reduced cardiac output, however, did not develop diffuse cardiac fibrosis (manuscript in preparation).

We have established novel methods for in vivo and ex vivo analyses of renal functional vasculature and capillary rarefaction in mice (2, 76) and confirmed our results in a translational approach (8) (Figure 1).

We have also analyzed the expression and regulation of PDGF ligands and receptors in the cardiac tissue of these CKD models and the role of PDGF-C in capillary rarefaction in CKD (1). In line with this, we additionally analyzed the role of PDGF-D in renal fibrosis (6).

We have extended our analyses in several fruitful cooperative works, i.e. with Dr. Krusche we have examined the regulation of PDGFs in hearts of Dsg2 deficient mice with cardiomyopathy; together with Dr. Stoppe and Dr. Götzenich we have measured PDGFs in cardiac cell culture under in vitro modeled uremic conditions as well as the influence of MIF in acute organ damage (9 and manuscript under revision), and together with Dr. Lehrke we have analyzed the role of GLP-1 in metabolic kidney and heart disease (Award for top abstract at the ERA-EDTA 53rd Congress, Vienna; manuscript in revision). Finally, we also investigated novel diagnostic approaches in progressive kidney diseases (3, 9).

Figure 1: Scanning electron micrographs (EM) of clots prepared from pooled fibrinogen of A: healthy control individuals, B: hemodialysis patients, C: purified fibrinogen which was not modified, D: in vitro guanidinylated fibrinogen *** p<0.001.
Using desmoglein 2-mutant mice as models of arrhythmogenic (right ventricular) cardiomyopathy, a desmosome-related hereditary disease of unknown pathogenesis, the following questions were addressed:

I. What triggers cardiomyocyte death?
   Focal mechanical stress was detected in juvenile hearts by studying the distribution of mechanical stress markers.

II. What roles do inflammation, fibrosis, and metabolism play in progression?
   - acute aseptic inflammation occurs during disease onset followed by chronic elevation of cardiac macrophages and T cells,
   - expression of MMP and protease inhibitors is increased (Fig. 1 A),
   - mRNA and protein expression of effector glucose and fatty acid metabolism are altered.

Based on the results to point II we also investigated, whether intercalated discs are structurally and compositionally altered in uremic and diabetic cardiomyopathy.

Third-party funding (DFG, BMBF, EU, foundations)

<table>
<thead>
<tr>
<th>Project</th>
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<th>Staff</th>
<th>Grant amount</th>
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<tr>
<td>Boor, P./Fleige, J. PDGF-a mediator of CVD in CKD</td>
<td>01/2018-12/2021</td>
<td>€ 449,000</td>
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<td>Boor, P./Hohl</td>
<td>Central platform for standardization and development of animal models and histopathological analyses</td>
<td>01/2018-12/2021</td>
<td>€ 630,400</td>
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<td>Boor, P. Kidney keratins - endogenous cytoprotective factors?</td>
<td>07/2017-06/2019</td>
<td>€ 307,800</td>
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<tr>
<td>Boor, P./Lungerich, T./Franklin, B. Analyses of Hepatic and Renal Fibrosis</td>
<td>01/2017-12/2020</td>
<td>€ 486,400</td>
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<tr>
<td>Boor, P./Fleige, J. Cells-specific role of PDGF system in renal fibrosis</td>
<td>01/2017-12/2020</td>
<td>€ 486,400</td>
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<tr>
<td>Boor, P. Heisenberg Professor – Translational Nephropathology</td>
<td>2017-2020</td>
<td>€ 376,200</td>
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Awards

2017 – Carl Ludwig Award of the German Society of Nephrology
2017 – Gloria Gallo Award of the Renal Pathology Society (RPS)
2018 – RWTH fellow (JF), award for excellent professors at the RWTH Aachen University

Publications


Figure 1: Summary of pathological alterations detected in different cardiomyopathies. A) Matrix metalloproteinase (MMP) and MMP inhibitor mRNA expression was elevated during fibrotic myocardial remodelling in Dsg2 mutant mice, a model of arrhythmogenic cardiomyopathy.

B) Connexin 43 (Cx43) mislocation was shown to be a hallmark in uremic cardiomyopathy induced by an adenine rich diet in wildtype mice (white arrow: intracellular Cx43 containing vesicles; white triangles: Cx43 mislocated at the lateral cell membrane).

C) Impaired mRNA expression of ICD proteins was unraveled in a murine model of diabetic cardiomyopathy (db/db mice) indicating that metabolism and structural gene expression is interconnected.

Applied third-party funding (DFG, BMBF, EU, foundations)

<table>
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<th>Project Title</th>
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<th>Institution</th>
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<th>Status</th>
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<tr>
<td>High-throughput Assessment of therapeutic molecules for arrhythmogenic right ventricular cardiomyopathy (ACCORD)</td>
<td>Krusche, C. A.</td>
<td>RWTH Aachen, Faculty 10</td>
<td>€ 242,292</td>
<td>Rejected</td>
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<td>Transnational Research Projects for Innovative Therapeutic Approaches for Rare Diseases</td>
<td>Leube, R. E.</td>
<td>RWTH Aachen, Faculty 10</td>
<td>€ 242,292</td>
<td>Rejected</td>
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</table>

Promoting of young researchers

**Doctoral Theses**

- **Gercek, Mustafa**, 2017 RWTH Aachen, Faculty 10: Ventriclespecific cardiac hypertrophy in ARVC – studies in patients and Dsg2 mutant mice
- **Lawin, D.**, 2017 RWTH Aachen, Faculty 10: Comparative analyses of structure and protein composition of intercalated discs in uremic, diabetic and desmoglein 2 mutant hearts
- **Bruns, F.**, Ongoing RWTH Aachen, Faculty 10: Compensatory mechanisms in the hearts of uremic, diabetic and desmoglein 2 mutant mice
- **Gercek, Muhammed**, Ongoing RWTH Aachen, Faculty 10: Analysis of cardiac MMP and MMP inhibitor expression in desmoglein 2 mutant mice – a murine model for arrhythmogenic cardiomyopathy
- **Kienste, T.**, Ongoing RWTH Aachen, Faculty 10: Study on the effects of Metoprolol treatment on cardiac function, hypertrophy and fibrosis in desmoglein 2 mutant mice
- **Lubos, N.**, Ongoing RWTH Aachen, Faculty 10: Studies on the mechanisms leading to structural disease onset in ARVC mouse models
Role of the plasma protein fetuin-A in lipidic calcified debris metabolism

Jahnen-Dechent, W.
(Helmholtz-Institute for Biomedical Engineering – Biointerface)

The plasma protein fetuin-A is a systemic inhibitor of calcification. Fetuin-A stabilizes protein-mineral complexes in the form of calciprotein particles (CPPs) and mediates their clearance from the circulation through scavenger receptor A. Reduced serum levels of free fetuin-A and increased concentrations of circulating CPPs are associated with increased cardiovascular mortality in CKD patients.

Our work suggests that CPPs bind to lipoproteins to form lipidic calcified debris, which we hypothesize to cause inflammation and calcification in CKD. Thus fetuin-A may become quintessential to maintain tissue homeostasis in conditions of both mineral disturbance as well as dyslipidemia, typical of multimorbid CKD/CVD patients.

We study the role of fetuin-A in the formation, transport and clearance of calciprotein particles and lipidic calcified particles (LCPs). Therefore, we apply biochemical/biophysical methods of colloid chemistry, cellular studies of uptake, inflammatory and calcifying potential of CPPs and LCPs, as well as mouse models of combined CKD/CVD and defective clearance.

Proteomic analyses of synthetic CPPs from healthy volunteer and CKD patient plasma samples performed in collaboration with the IZKF proteomics facility and the IZKF Research Group Bioinformatics demonstrates a strong relative concentration of fetuin-A in CPPs underscoring the role of fetuin-A in calcified matrix metabolism. Proteomic data also revealed high abundance of apolipoproteins and components of intrinsic blood coagulation in these particles further indicating the existence of mixed lipidic calcified particles (Figure 1).

The 100 most abundant proteins in CPP preparations showed a protein clustering according to their origin of primary vs. secondary CPPs and a sub-clustering into control and patient groups. This suggests that disease-specific plasma protein markers are present in CPPs from dialysis patients. (Figure 1).

T50 assays of postprandial lipemic serum compared to fasting serum showed no significant difference in the T50 time, but displayed a decline in turbidity instead of an expected increase. Normal T50 curves show a specific increase in turbidity while ripening of primary to secondary CPPs occur. In lipemic serum initial turbidity is much higher and the specific increase is inverted, suggesting lipid binding during CPP ripening. Binding of lipid to secondary CPPs could also be shown in electron microscopy pictures of CPPs from postprandial lipemic serum compared to fasting CPPs (Figure 1).

In conclusion, our results demonstrate the existence of LCPs and their role in inflammation and calcification. Further research will clarify their exact composition and biological activity.
From cells to animals and back: an integrated optical imaging platform for cardiovascular research

van Zandvoort, M. (Biophysics of Microscopy, Institute for Molecular Cardiovascular Research IMCAR)
Kiessling, F. (Institute for Experimental Molecular Imaging, Helmholtz Institute for Biomedical Engineering)

Within the funding period, two peer-reviewed publications in the top 10% journals of its respective field were achieved. Within the scope of the project, fluorescently labelled microbubbles were applied on cardiovascular research. We combined clinical ultrasound with two-photon microscopy to generate an optical imaging platform using the bi-modal contrast agent in order to characterize/monitor endothelial dysfunction in vitro, ex vivo and in vivo (from cells to animals). Two cardiovascular markers were established and published: 1. VCAM-1 was discovered as a surface marker for endothelial injury and regeneration, with great potential to be used for clinical monitoring of endothelial regeneration after intervention. 2. JAM-A surface redistribution during flow-dependent endothelial activation was characterized and exploited to detect early onset of plaque development. Both publication contribute greatly to the development of a new generation of cardiovascular contrast agents. Additionally, we are in the early (in vitro) stage of a third manuscript, investigating the function of riboflavin receptors during endothelial inflammation and its potential for drug targeting. Using our optical imaging platform we serviced and collaborated with members of the IZKF cluster, i.e. super-resolution structural studies of molecular contrast agents (Department of Experimental Imaging) and deep tissue imaging of calcification (Department of Biomedical Engineering).

Publications
Influence of C-peptide on cardiac metabolism and function in ischemia/reperfusion injury

Marx, N. (Department of Internal Medicine I)
Lebherz, C. (Department of Internal Medicine I)

Patients with type 2 diabetes are at an increased risk for the development of cardiovascular diseases. In the early phase of insulin resistance the metabolic phenotype is characterized by hyperinsulinemia and increased c-peptide serum levels. Positive associations of increased c-peptide levels with a worse cardiovascular outcome have been documented in clinical studies. Even though a causal relationship has not been proven, several in vitro data confirm a pro-apoptotic and pro-inflammatory c-peptide effect. In addition, C-peptide treatment promotes atherogenesis in mice. We therefore wanted to evaluate, if there exists a direct link between c-peptide concentration and cardiac function after myocardial infarction.

In our study acute treatment of non-diabetic mice with c-peptide surprisingly resulted in an improved contractility 6 h post myocardial infarction via increased GSK3 phosphorilation (inhibition). Long term treatment of these mice using an AAV vector system in contrast did not result in any significant effects on cardiac function after myocardial infarction. In vitro studies in cardiomyocytes verified c-peptide dependent GSK3 phosphorylation (=inhibition) which was mimicked under insulin resistant conditions suggesting different c-peptid effects depending on the metabolic milieu.

In conclusion, we were able to show that c-peptide has acute cardioprotective effects in rodents whereas long-term c-peptide treatment does not improve cardiac function in non-diabetic mice. Further studies are needed to clarify the c-peptide effects in the setting of acute or chronic myocardial infarction in the context of hyperglycemia/hyperinsulinemia.

Promoting of young researchers
Doctoral Theses

Balanu-Curaj, A. 2017 Maastricht University, CARM Molecular ultrasound imaging – a powerful tool for the diagnosis of endothelial dysfunction and arterial inflammation
Novel exacerbating factors in myocardial ischemia/reperfusion

Stoppe, C. (Department of Intensive Care Medicine)  
Goetzenich, A. (Department of Thoracic & Cardiovascular Surgery)

During myocardial ischemia/reperfusion (I/R), cytokines, hormones and growth factors are released and mediate the following inflammatory response, which may lead to the development of organ dysfunctions, such as acute kidney injury. Macrophage migration inhibitory factor (MIF) was already shown to play a role in many chronic inflammatory diseases, such as atherosclerosis, and acute inflammatory diseases, such as sepsis. Interestingly, besides its predominant pro-inflammatory characteristics, previous studies indicate, that MIF is secreted following myocardial I/R and provides cardioprotective properties, which are mediated by the regulation of different signalling pathways, and its antioxidant capacity. In addition, a close functional homolog to MIF was recently found, called D-dopachrome tautomerase (or MIF-2), which has been intensively investigated by our group (published in 2015, Stoppe C et al ARS 2015). To characterize the kinetics of both cytokines in the setting of myocardial I/R, serum concentrations of cardiac surgery patients were measured and the clinical relevance evaluated. We found, that elevated MIF levels during myocardial reperfusion are independently associated with a significantly reduced risk for the occurrence of atrial fibrillation (AF) in the postoperative period. In contrast, we revealed that MIF-2 mainly provides disease aggravating properties (e.g. leading to the development of atrial fibrillation in patients exposed to myocardial I/R). We provide first evidence about a renoprotective role for MIF in AKI patients, after cardiac surgery, by reducing regulated necrosis and mitigating oxidative stress in renal tubular epithelial cells (Stoppe C, Averdunk L et al, under review).

We highlight a new signaling pathways, that sCD74/MIF axis in myofibroblasts promotes an antifibrotic pathway by redirecting the MIF-activated CXCR4/AKT pathway to a CD74-dependent necroptotic pathway. First data provided evidence about the clinical relevance of sCD74/MIF ratio for progression of heart failure (Soppert J, et al, Stoppe C., under review Circulation Research 2018).

Publications
Soppert, Stoppe et al., „Soluble CD74 reroutes MIF/CXCR4/AKT-mediated survival of cardiac myofibroblasts to necroptosis“. Circulation research (submitted 2017, under review) [IF 13.9]
Soppert C, Averdunk L et al P Boor:  The protective role of macrophage migration inhibitory factor (MIF) in acute kidney injury after cardiac surgery (submitted 2017, under review) [IF 16.0]

Applied third-party funding (DFG, BMBF, EU, foundations)
Stoppe, C. In preparation/associated to the publication under review at Science Translational Medicine “The protective role of macrophage migration inhibitory factor (MIF) in acute kidney injury after cardiac surgery”
Stoppe, C. DFG grant (GZ: STO 1099/2-1): “Die Rolle von MIF innerhalb der kardialen ischämischen Konditionierung“

Promoting of young researchers
Doctoral Theses
Soppert, J. 2017 RWTH Aachen, Faculty 1 The Functional Role of the Macrophage Migration Inhibitory Factor Protein Family in Myocardial Fibrosis
Emontzpol, C. 2017 RWTH Aachen, Faculty 1 Influence of myocardial I/R on the inflammatory response and MIF-mediated EPC mobilization
Averdunk, L. 2018 RWTH Aachen, Faculty 10 The protective role of macrophage migration inhibitory factor (MIF) in acute kidney injury after cardiac surgery

Appointments to other universities
Goetzenich, A. Aibamed, Europe, European lead R&D W2 equivalent accepted
The Board decided to end the project funding ahead of schedule in 2016.

**N7-6** | Gründer, G.
Modifying pathological aggression: induction, pathology and treatment under the scope of dopaminergic and serotonergic transmission

**N7-7** | Habel/Beyer
Effects of testosterone and arginine vasopressin on the plasticity of neural aggression networks: gender aspects and comparability of animal and human models

**N7-8** | Binkofski/Mottaghy
Modulation of the dopaminergic fronto-striatal network function by non-invasive brain stimulation and cognitive-affective stimulation

**N7-9** | Freiherr
Plasticity of the visual cortex in ischemic stroke patients
Alterations in connectivity have a major role in the pathophysiology of neurodegenerative and neurological diseases as well as psychiatric disorders. Alterations can be identified on different levels including the molecular, cellular, and systemic level. The different projects concentrated on the pathogenesis of amyotrophic lateral sclerosis (ALS) and Parkinson’s disease (PD), investigating the role of α-synuclein and dopamine as well as misfolded proteins. Systemic research on connectivity tested the influence of Transcranial magnetic stimulation (TMS) on neural connectivity. Moreover functional brain connectivity underlying multisensory processes as well as the integration of hormonal mechanisms and neural connectivity in aggression were investigated. On a molecular level, it was shown that clearance of α-synuclein aggregates depends on CDK16 induced autophagy. Further, regarding the cellular mechanisms of α-synuclein, a project revealed that cell-to-cell transfer of α-synuclein is dependent on Rab11 and transmission depends on neuronal activity. Additional results contributing to the understanding of PD demonstrated that the loss of dopaminergic neurons within the SNpc would be paralleled by a cortical dopaminergic denervation in the 6-OHDA model. On the cellular level, a major progress was also the establishment of a microfluid system in which cortical and striatal neurons are separated and of a cell model for a human dopaminergic neuron, the LUMES cell. Lastly, investigating protective factors in ALS, it was found that alterations of ER chaperone proteins are leading to progressive degeneration of neuromuscular axons where they impair neuronal connectivity. In addition, aggregates are transmitted in axons where they impair neuronal connectivity.

Systemic projects showed that testosterone administration causally influenced aggressive behaviour in mice and humans by acting on brain structure (organizational effects) and functional connectivity (activation effects). Influences on connectivity were also analyzed with respect to intermittent theta burst stimulation. Fronto-striatal functional connectivity in the human brain was evaluated and modulated by different intensities of TMS or TDCS stimulation. FMRI imaging of multimodal olfactory-visual perception revealed a network of functional connectivity changes depending on the congruence of the presented stimuli. Inferior frontal gyrus plays a major role in this network.

In Parkinson disease, aggregates of α-synuclein accumulate in neuronal somata and along axons where they impair neuronal connectivity. In addition, aggregates are transmitted from neuron to neuron and spread from one brain area to the next. Autophagy is a cellular defence strategy in which cytosolic components are engulfed by a vesicle. This autophagosome can then fuse with lysosomes to degrade its content. As we demonstrated in the course of this project, autophagosomes may alternatively acidify without fusion to lysosomes, be transported to the plasma membrane and likely secreted, linking autophagy as a pathway for aggregate degradation to pathways leading to aggregate transmission. In this project we have investigated the impact of CDK16 for autophagy and aggregate clearance. We have biochemically characterised the upstream signalling cascade that activates CDK16. This cascade includes AMPK, which was recently demonstrated to mediate the neuroprotective effects of calorie restriction in an animal model of Parkinson disease (Bayliss et al., J. Neuroscience 2016). Specifically, we have demonstrated that AMPK phosphorylates cyclin Y at Serine 326 and is necessary for the interaction of cyclin Y with its cyclin-dependent kinase CDK16. This interaction induces autophagy as demonstrated by biochemical methods (LC3 lipidation) and imaging (LC3 dot formation). In addition we have demonstrated that autophagy induced by cyclin Y-CDK16 leads to clearance of α-synuclein aggregates in cultured cells as demonstrated by biochemical α-synuclein measurements, bimolecular fluorescence complementation and imaging and rescues a locomotor defect in an α-synuclein driven neurodegenerative fly model. In order to further test the relevance of the CDK16 pathway in vivo we screened an inhibitor library in cooperation with the Lead discovery Centre in Dortmund. The screening revealed six lead compounds, which significantly inhibits the activity of cyclin Y/CDK16 in vitro and in cells and which will be first tested in the fly model.

Publications


Habel, U. (Department of Psychiatry, Psychotherapy, and Psychosomatics)

In this project we have investigated the impact of CDK16 for autophagy and aggregate clearance. We have biochemically characterised the upstream signalling cascade that activates CDK16. This cascade includes AMPK, which was recently demonstrated to mediate the neuroprotective effects of calorie restriction in an animal model of Parkinson disease (Bayliss et al., J. Neuroscience 2016). Specifically, we have demonstrated that AMPK phosphorylates cyclin Y at Serine 326 and is necessary for the interaction of cyclin Y with its cyclin-dependent kinase CDK16. This interaction induces autophagy as demonstrated by biochemical methods (LC3 lipidation) and imaging (LC3 dot formation). In addition we have demonstrated that autophagy induced by cyclin Y-CDK16 leads to clearance of α-synuclein aggregates in cultured cells as demonstrated by biochemical α-synuclein measurements, bimolecular fluorescence complementation and imaging and rescues a locomotor defect in an α-synuclein driven neurodegenerative fly model. In order to further test the relevance of the CDK16 pathway in vivo we screened an inhibitor library in cooperation with the Lead discovery Centre in Dortmund. The screening revealed six lead compounds, which significantly inhibits the activity of cyclin Y/CDK16 in vitro and in cells and which will be first tested in the fly model.

CDK16 signalling regulates autophagy and release of protein aggregates

Falkenburger, B. (Department of Neurology)

Vervoorts, J. (Institute of Biochemistry and molecular Biology)

Funding period: 01.07.2014 - 31.12.2017

Staff: TVL-13 (65%) Dohmen, M, respectively TVL-L13 (50%) Sandlak, T.

Total Budget 2017: € 513,009
The role of acid-sensitive ion channels in transmission at a cortico-striatal synapse.

Gründer, S. (Institute of Physiology)
Falkenburger, B. H. (Department of Neurology)

The principal aim of this project was to establish a co-culture of cortical and striatal neurons, in which they form glutamatergic synapses, and to use this co-culture to study the role of acid-sensing ion channels (ASICs) for synaptic transmission. We succeeded in establishing the co-culture; we also established protocols to routinely distinguish between cortical and striatal neurons via their electrophysiological properties and between different medium spiny neurons (MSNs) from the striatum via their response to dopamine receptor agonists (D1 or D2). This allowed us to characterize ASICs in different subpopulations of MSNs and in mono-culture vs. co-culture. Functional and pharmacological characterization suggests that the ASIC current in MSNs is mainly carried by the homomeric ASIC1a subtype. Moreover, we found a trend for smaller ASIC currents (total currents and current density) in co-culture as compared to monoculture. These results are currently prepared for publication.

To study synaptic transmission in the co-culture, we established a microfluidic system in which cortical and striatal neurons are physically separated. This allows, in principle, to excite one neuron (e.g. the cortical neuron) and record from the other neuron (e.g. from the MSN). We successfully used Ca2+ imaging to record postsynaptic responses, but due to different technical problems, we so far did not succeed in recording postsynaptic responses by more sensitive electrophysiological recordings.

Another aim of our study was to study dopaminergic modulation of the cortico-striatal synapse. As we could not routinely record synaptic transmission, we first established a cell model for a human dopaminergic neuron, the LUHMES cell, and characterized ASICs in these neurons. LUHMES cells also allowed us to investigate the influence of ASICs on differentiation of dopaminergic neurons. These results are also currently prepared for publication. In the future, LUHMES cells can be used together with cortical and/or striatal neurons, also in microfluidic systems, to study the dopaminergic influence on these neurons.
Figure 1: Characterization of medium spiny neurons from the striatum. Left, distribution of D1 and D2 dopamin receptors in mono-culture vs. co-culture. In co-culture, there are significantly more neurons responding to D1 and D2 agonists together than in monaculture and the number of neurons responding only to the D2 agonist is significantly reduced ($\chi^2$ test). Right, current densities of ASIC currents elicited by different pH in mono- vs. coculture (mean +/- SD). The ASIC current density is not significantly different in mono-culture vs. co-culture (2-way ANOVA).

Promoting of young researchers

Bachelor/Master Theses

Engels, J. 2017 RWTH Aachen, Faculty 1  Charakterisierung kortikaler, striataler und dopaminerge Neurone in Mono- und Ko-Kultur

Doctoral Theses

Neuhof, A. Ongoing RWTH Aachen, Faculty 1  Working title “The role of acid-sensitive ion channels in transmission at a cortico-striatal synapse”

Postdoctoral lecture qualification

Falkenburger, B. Habilitation in 2017 RWTH Aachen, Faculty 10  Signaling by plasma membrane receptors and phospholipids in neurologic diseases

Cell-to-cell transfer of $\alpha$-Synuclein

Voigt, A. (Department of Neurology)
Schulz, J. B. (Department of Neurology)

We successfully reached all aims claimed in our proposal.

1. We generated the first in vivo system allowing mechanistic analysis of $\alpha$-Synuclein cell-to-cell transfer.

2. We identified gene products/cellular mechanisms involved in cell-to-cell transfer of $\alpha$-Synuclein. Here, we were able to show that transmission is dependent on Rab11. Thus, $\alpha$-Synuclein is at least partially released via the exocytotic pathway in flies. Unfortunately, our current model system does not allow genetic modification of the receiving neuron.

3. We were able to show that transmission is dependent on neuronal activity. Inactive neurons do not (have strongly reduced) release of $\alpha$-Synuclein.

4. We provide strong evidence that $\alpha$-Synuclein oligomers are transmitted from cell-to-cell. In line with our data, it is very unlikely that monomeric $\alpha$-Synuclein is transmitted

5. We identified and analysed factors that were suggested to contribute to $\alpha$-Synuclein aggregation and/or the removal of aggregated $\alpha$-Synuclein in vivo.

Funding period:  01.07.2014 - 31.12.2017

Staff:
TV-L13 (65%):  Prasad, V.
Promoting of young researchers

Doctoral Theses

Prasad, V.  
Ongoing  
RWTH Aachen, Faculty 1  
Cell to Cell Transfer of alpha-Synuclein in an in vivo model system

Figure 1: Preparations of adult fly brains stained for pigment-dispersing factor (pdf) in red. Pdf is a secreted, biologically active neuropeptide that acts via a specific G protein-coupled receptor to trigger intracellular signaling. Pdf has a prominent role in the physiology of circadian rhythms. Accordingly dendritic projections of pdf neurones reach the optic lobes (ol) where visual input is processed. The pdf expressing neurones also express human α-Synuclein (green). White circles indicate pdf-negative, but α-Synuclein positive neurones. However, these neurones do not express α-Synuclein. Thus, transmission of α-Synuclein from the pdf-neurones to these neurones is likely. We were able to provide strong evidence for this assumption as 1st GFP expressed in pdf neurones is not transmitted to receiving cells, 2nd neuronal activity of the pdf neurones is required for transmission (flies raised in the dark do not show transmission) and 3rd depletion of Rab11 from pdf neurones is sufficient to block transmission of α-Synuclein.

Visualization of α-Synuclein is facilitated using bimolecular fluorescent complementation. Accordingly only aggregated α-Synuclein emits green fluorescent signals (α-Synuclein dimers represent smallest functional fluorescent emitting unit).

Sigma receptor 1 chaperonopathy and loss of connectivity in ALS and Parkinson’s disease

Goswami, A. (Institute of Neuropathology)  
Weis, J. (Institute of Neuropathology)

Misfolded proteins which often form intracellular aggregates are the pathological hallmark of amyotrophic lateral sclerosis (ALS) and related neurodegenerative disorders. ALS is characterized by selective degeneration of motor neurones (MNs) and their target muscles. Alterations and disruption of the functional interplay between protein degradation (ubiquitin proteasome system and autophagy) and RNA binding protein homeostasis has recently been suggested as an integrated model that merges several ALS-associated / aggregate prone proteins into a common pathophysiological pathway.

Within this line of research and during the current funding period, we investigated the involvement of the endoplasmic reticulum (ER) chaperones Sigma receptor 1, SIL1 and VAPB and their co-relation with several ALS associated RNA binding proteins including TDP43, FUS and Matrin-3 in ALS and related disorders such as peripheral neuropathy. Our recent work suggests that these ER chaperone proteins are actively involved in these diseases, provide neuroprotection and serve important ER-associated functions. Alterations of these protective factors lead to progressive degeneration of neuromuscular axis (MN cell bodiess, axons and skeletal muscles) and finally to altered connectivity. Pharmacological manipulation of these and related proteins might inhibit progression of ER pathology simultaneously in both muscle fibers and MNs. This way, it should ameliorate MND and related disorders by targeting the disease process at multiple levels.

Publications

[IF 12.1]


Funding period: 01.07.2014 - 31.12.2017

Staff:
TV-Ä 1 (50%): Nolte, K.
TV-L 13 (50%): Tripathi, P.


*Equal contribution

Third-party funding (DFG, BMBF, EU, foundations)

Weis, J./Katona, I./Knüchel-Clarke, R./Hermanns, B./Leube, R.

Weis, J./Katona, I.

Weis, J.

Weis, J./Gaswani, A.

Weis, J./Gaswani, A.

Promoting of young researchers

Doctoral Theses

Volbracht, J. T.

Jesse, C. M.

Yamash, A.

Ongoing
Deep brain stimulation for Parkinson’s disease: from neuroprotection to neuronal connectivity

Beyer, C. (Institute of Neuroanatomy)
Tan, S. (Department of Neurosurgery)

Parkinson’s disease (PD) is a neurodegenerative disorder characterized by progressive loss of midbrain dopaminergic neurons, resulting in motor and non-motor symptoms. The underlying pathology of non-motor symptoms is poorly understood. Discussed are pathological changes of extrastriatal brain structures. In this study, we characterized histopathological alterations of extrastriatal brain structures in the 6-hydroxydopamine (6-OHDA) PD animal model. Lesions were induced by unilateral stereotactic injections of 6-OHDA into the striatum or medial forebrain bundle of adult male mice. Loss of tyrosine hydroxylase positive (TH+) fibers as well as glia activation was quantified following stereological principles. Loss of dopaminergic innervation was further investigated by western-blotting. As expected, 6-OHDA injection into the nigrostriatal route induced retrograde degeneration of dopaminergic neurons within the substantia nigra pars compacta (SNpc), less so within the ventral tegmental area. Furthermore, we observed a region-specific drop of TH+ projection fiber density in distinct cortical regions. This pathology was most pronounced in the cingulate- and motor cortex, whereas the piriform cortex was just modestly affected. Loss of cortical TH+ fibers was not paralleled by microglia or astrocyte activation. Our results demonstrate that the loss of dopaminergic neurons within the SNpc

is paralleled by a cortical dopaminergic denervation in the 6-OHDA model. This model serves as a valuable tool to investigate mechanisms operant during cortical pathology in PD patients. Further studies are needed to understand why cortical dopaminergic innervation is lost in this model, and what functional consequence is associated with the observed denervation.

Publications

Funding period: 01.07.2014 - 31.12.2017
Staff: TV-L13 (50%): Becker, B.

Figure 1: Injections of 6-OHDA in the striatum lead to pathological changes in both nigrostriatal and extra-nigrostriatal structures.

Promoting of young researchers
Doctoral Theses

Becker, B. Ongoing RWTH Aachen, Faculty 10 Cortical pathologies and astrocyte heterogeneity in rodent models of multiple sclerosis and Parkinson’s disease
Effects of testosterone and arginine vasopressin on the plasticity of neural aggression networks: gender aspects and comparability of animal and human models

Habel, U. (Department of Psychiatry, Psychotherapy, and Psychosomatics)
Beyer, C. (Institute of Neuroanatomy)

The project focused two research lines testing the effect of exogenous testosterone (T) on aggressive behavior.

1) A mouse model, which included social deprivation and T substitution for 3 weeks, was investigated in terms of aggressive behaviors to intruders in the Institute of Neuroanatomy. The results showed that mice that were substituted with T demonstrated more keeping down behavior and lateral threat behavior than socially deprived control mice. In addition the expression of the androgen receptor in the cerebral cortex of T substituted mice was reduced.

2) The human experiment investigated the effect of T on the plasticity of neural aggression networks applying a double-blind placebo controlled design. Effects of exogenous T additionally were tested for interactions with genetic variability investigating the monoamine oxidase A polymorphism. The polymorphic region with a lower number of tandem repeats (MAOA-S) had previously been identified as a sex specific vulnerability factor of increased aggression in humans. The project demonstrated that exogenous T reduced the avoidance (personal distance) of aggressive counterparts. Importantly, similar to the animal model T increased the reactivity towards social and non-social provocation in terms of anger and aggression and amygdala reactivity when being provoked. An interaction of testosterone and the MAOA polymorphism was ascertained for risk-taking behavior, which was increased via T only in MAOA-S carriers. Interestingly, aggression was reduced in MAOA-S carriers after T administration which was accompanied by an increased functional connectivity between amygdala and the medial prefrontal cortex.

Both projects demonstrate that the male steroid hormone T exerts a strong effect on aggressive behaviors in humans and mice. Underlying neural plasticity is demonstrated by modulations of androgen receptors, functional brain activity and connectivity that points towards a complex interaction of T and the monoaminergic system.

Currently 3 manuscripts have been published, 3 manuscripts are under review and one manuscript is in preparation.

Funding period: 01.07.2014 - 31.12.2017
Staff: TV-L13 (50%): Frintop, L., Wagels, L.

Publications

Awards
Poster prize (300 Euro) at the conference of the DGPA (German Society for Psychology and Applications). Title: Ich komme trotzdem! Testosteron im Blut macht Männern Mut.
Modulation of the dopaminergic fronto-striatal network function by non-invasive brain stimulation and cognitive-affective stimulation

Binkofski, F. (Section Clinical-Cognitive Sciences)
Mottaghy, F. (Department of Nuclear Medicine)

The main goal of our IZKF project was the evaluation of functional connectivity between the dorso-lateral prefrontal cortex (DLPFC) and the basal ganglia (BG). The crucial experiment was the transcranial magnetic stimulation (TMS) of DLPFC and measurement of resulting changes in postsynaptic dopaminergic effects in the BG using dynamic Desmethoxyfallypride-PET. Since the project proposal (submitted early in 2015) to the federal office for radiation protection (BfS) was approved in beginning of 2017, we just recently finished the PET measurements and are currently performing the complex data analysis. We expect to present the results to IZKF in due time. One exemplary Fallypride-PET pre- and post-TMS stimulation in one subject is presented in Figure 2.

Meanwhile we performed magnetic resonance imaging (MRI) measurements following TMS to DLPFC. By this we measured the resulting changes in BG functional connectivity (FC) using functional MRI (fMRI). We measured differential modulation effect of TMS at different intensities to assess the optimal stimulation strength. Directly before and after stimulation, task-free resting state fMRI was conducted. It was found that sub-threshold-iTBS increased FC directly in between the left DLPFC and the left caudate (lCA), as well as indirectly between the striatal sub regions (right caudate (rCA), nucleus accumbens (NA) and putamen (PU)) of both hemispheres. Supra-threshold stimulation decreased FC between the DLPFC and the two CA bodies, respectively, and increased FC between the rCA and the right-sided amygdala (AMr). This paradoxical effect is possible due to homeostatic mechanisms in form of increased surround inhibition in DLPFC induced by higher TMS strengths (Figure 1). These results were not only relevant for the recently finished PET study, but also for further research entailing TMS to the prefrontal cortex, as well as for therapeutical applications. We were able to demonstrate that different stimulation intensities can have a vastly different, nonlinear effect on network function. The results were presented at two conferences and a corresponding research paper is under revision in the Journal “Brain Structure and Function”.

In addition, we performed another related experiment in which the DLPFC was stimulated, this time by combination of TMS and direct current stimulation (tDCS). Here tDCS was used to get a so-called pre-conditioning for the TMS stimulation. Functional connectivity between the DLPFC and the BG was measured pre- and post-stimulation by means of resting state fMRI. This study was funded by the START program of our Medical Faculty. The data acquisition is finished and the data analysis is being performed at the moment.
Plasticity of the visual cortex in ischemic stroke patients

Freiherr, J. (nee Albrecht) (Diagnostic and Interventional Neuroradiology)

Regarding the original proposed project, we were able to develop and optimize experimental and analytical procedures allowing us to investigate performance on visual perception tasks and measure functional activation and connectivity using fMRI measurements. The prevalence of viable patients was, however, significantly lower than expected. As a result of this, we were unable to recruit the number of patients required to obtain enough data for meaningful analyses. Ethical approval for the project has been extended to the end of 2018 to allow for a potential follow-up on the project.

As olfactory performance of patients and the influence of disrupted neural connections was part of the original hypothesis, the employed PhD student conducted an additional fMRI project investigating a functional network, revealing differences in functional connectivity depending on the level of congruence of visual and olfactory stimuli presented to research participants. The manuscript is currently under revision at the journal Chemical Senses.

Related to this project, we optimized an existing psychophysical method of measuring one’s olfactory function as determined by olfactory perceptual threshold. This new procedure significantly reduces time required to obtain this olfactory sensitivity measures and reduces the participant’s perceptual strain. Results of this project have been published in the journal Chemosensory Perception and are already being used by multiple research groups.

Publications

Promoting of young researchers
Doctoral Theses
Sijben, R. Ongoing RWTH Aachen, Faculty 10 Psychophysiology, neural processing, and functional connectivity of olfactory and visual perception. (working title)

Publications

Promoting of young researchers
Doctoral Theses
Sijben, R. Ongoing RWTH Aachen, Faculty 10 Psychophysiology, neural processing, and functional connectivity of olfactory and visual perception. (working title)
E7 | JOINT RESEARCH PROJECT Floege p. 92
Decisions in inflammation

E7-1 | Tacke p. 93
Molecular determinants of functional divergent monocyte/macrophage subset differentiation in hepatic inflammation

E7-2 | Floege/Ostendorf p. 95
IL-6 classical signalling versus trans-signaling in inflammatory renal diseases

E7-3 | Trautwein/Liedtke p. 97
Role of Gp130 for cellular decisions between transient inflammation and uncontrolled hepatocyte growth

E7-4 | Tenbrock p. 99
Do CREM/CREB mediated pathways regulate pro and anti-inflammatory properties of macrophages?

E7-5 | Uhlig/Reis p. 101
Inflammation and hyperinflammation in the lung

E7-6 | Borkham-Kamphorst/Weiskirchen p. 103
Lipocalin 2 (LCN2), a central mediator in inflammatory organ disease

E7-7 | Raffetseder p. 107
The role of YB-1 in reversible models of renal inflammation

E7-8 | Moeller p. 109
Tubular cells in acute kidney injury: repair or die

E7-9 | Ludwig/Ostendorf p. 111
Role of ADAM-family metalloproteinases for resolution and progression of lung and kidney inflammation

PROJECTS
Inflammation and Consequences
**Decisions in Inflammation**

**Floege, J. (Department of Internal Medicine II)**

Our group focuses on ‘sterile inflammation’, resulting from trauma, autoimmune reactions or physicochemical irritation, which often cannot be eradicated and develops towards organ failure. Here, decision mechanisms and consequences are poorly defined (Figure 1). The complexity of these processes cannot be comprehensively studied by individual laboratories but only in collaborative centers. The initiative is central to the research focus “Inflammation and Consequences” of the medical faculty. Besides the TRR57 Transregio (Aachen/Bonn) there is a high need of the Medical Faculty for a DFG funded consortium which is fully localized in Aachen. The faculty recruited new chairs of the Institutes of Molecular Medicine, Microbiology and Molecular Cardiology that strengthen inflammation research at the RWTH Aachen.

It was decided that the concept decisions of inflammation will not be directly developed and instead the focus will be on the „Gut liver axis“, which has now become a new DGF cluster (coordinator Mathias Hornef). The research on the gut liver axis is also central to a new SFB-initiative to characterize mutual functional changes in liver or gut in patients and animal models of intestinal or hepatic disorders and identify mechanistic cellular and molecular links between both organs during homeostasis and disease. In parallel a GRK-initiative “Systems Biology in Inflammation” has formed, which combines experimental inflammation research with analytical studies and bioinformatics.

**Molecular determinants of functional divergent monocyte / macrophage subset differentiation in hepatic inflammation**

**Tacke, F. (Department of Medicine III)**

Liver macrophages comprise ontogenetically and functionally different subsets. Tissue-resident Kupffer cells and infiltrating monocyte-derived macrophages are key players in the maintenance of immune homeostasis and regulation of inflammatory responses, respectively. During the DGF project, we have identified key signals that promote the recruitment and polarization of macrophage subsets in the liver, which determine their functionality in the context of liver injury. This is apparent in experimental models of acute (e.g., by acetaminophen poisoning) and chronic liver damage (e.g., experimental steatohepatitis). We employed an integrated approach, combining intravital multiphoton microscopy, multicolour FACS and transcriptomic profiling of sorted macrophage subsets (Figure 1). The different macrophage subsets can be therapeutically targeted. We have demonstrated that the application of an oral chemokine receptor CCR2/CCR5 inhibitor (cenicriviroc) effectively blocks monocyte infiltration in experimental steatohepatitis and thereby ameliorates liver fibrosis. Follow-up projects will further characterize hepatic macrophage heterogeneity using single-cell RNA sequencing analyses.

**Figure 1:** Macrophage subsets in hepatic inflammation. Left: Schematic picture of resident macrophages (termed “Kupffer cells”) and monocyte-derived macrophages in the liver. Right: These cells have different origin, differentiation and function, as evidenced by RNA-sequencing of Kupffer cells vs. monocyte-derived macrophages isolated from livers of mice that had been subjected to experimental steatohepatitis.
**Publications**


Promoting of young researchers

**Doctoral Theses**

**Peuschens, J.**<br>Ongoing<br>RWTH Aachen, Faculty 1<br>Macrophage heterogeneity in liver injury

**Niemetz, P.**<br>Ongoing<br>RWTH Aachen, Faculty 1<br>The effect of the chemokine receptor CCR7 on liver fibrosis development

**Applied third-party funding (DFG, BMBF, EU, foundations)**

**Tacke, F.**<br>Macrophages & chemokines in APAP liver failure<br>DFG, Tu345/1-5<br>11/2015-10/2018<br>€ 361,000

**Tacke, F.**<br>Monocyte and macrophage subsets in liver fibrosis<br>DFG, SFB/TR57, P09<br>01/2017-12/2020<br>€ 558,000

**IL-6 classical signalling versus IL-6 trans-signalling in inflammatory renal diseases**

**Floege J. (Department of Internal Medicine II)**<br>Ostendorf, T. (Department of Internal Medicine II)

Contradictory data exist on the interleukin (IL)-6/gp130 system in renal diseases. Pro- and anti-inflammatory effects have been described. A possible explanation is provided by two modes of IL-6 signalling: On the one hand, IL-6 binds the cell surface expressed IL-6 receptor (IL-6R) activating the signal transducer gp130 (= classical signalling mode). On the other, IL-6 can form a complex with soluble IL-6R and thereby activates gp130 on IL-6R-negative cells (= trans-signalling mode). Classical signalling mostly acts in an anti-, and trans-signalling mostly in a pro-inflammatory manner. In this project, we tested the hypothesis that IL-6 can improve or aggravate renal diseases depending on the predominant type of signalling. Our analyses demonstrated an immediate increase of renal IL-6 signalling with a delayed induction of the trans-signalling mode in patients with rapid progressive glomerulonephritis (RPGN), in an appropriate RPGN mouse model as well as in an LPS-induced acute renal inflammatory mouse model. By interventional studies in the RPGN mouse model we identified IL-6 trans-signalling as a key event in the progression of RPGN, pointing to new therapeutic options in this disease (Figure 1). We furthermore focussed on podocytes and parietal epithelial cells (PECs), which have been identified as key cells in the development of glomerular crescents during RPGN. Once activated, these cells migrate and proliferate. Expression analyses demonstrated that both cell types do not express the membrane-bound IL-6R, but the ubiquitous co-receptor and signal transducer gp130. Consequently, functional analyses clearly showed a susceptibility of these cells for IL-6 trans-signalling. This type of signalling, however, did not significantly increase survival or proliferation of these cells. In studies with genetically modified podocytes and PECs we finally identified other pathologically relevant effects of IL-6 trans-signalling, i.e. cell migration. Comparisons by microarray analyses of primary gp130 knock-out versus wildtype-podocytes detected differentially expressed genes, thereby pointing to a significant modulation of proteins of the cytoskeleton and cell adhesion factors by IL-6 trans-signalling.

**Publications**

Funding period: 01.07.2014 - 31.12.2017

**Ostendorf, T.** (Department of Internal Medicine II)

**Publications**

The goal of our project was to evaluate the consequences of constitutive, long term activation of the gp130 receptor in hepatocytes as found in patients with inflammatory hepatocellular adenomas. Based on the findings in patients, we hypothesized that constitutive activation of gp130 in mice should result in chronic liver inflammation and finally in liver cancer.

To test this hypothesis, we generated transgenic mice with inducible activation of a human, constitutive-active gp130 allele in hepatocytes taking advantage of the cre/loxP system (Fig. 1A). Efficient cre-induced gene expression of constitutive-active hgp130 was confirmed by quantitative real-time PCR. However, we also detected some hgp130 expression in cre-negative mice indicating that the inserted transgene is leaky and can be slightly expressed in absence of cre-recombinase (Fig. 1B). Importantly, in 16-week old transgenic mice we frequently detected inflammatory hepatic infiltrates and substantially increased spontaneous proliferation mainly of hepatocytes, but also of non-parenchmal liver cells suggesting that prolonged gp130 signalling can trigger pro-inflammatory signals and premalignant liver cell growth (Fig. 1C). However, long-term studies revealed that constitutive gp130 activation in mice did not lead to liver cancer development or other severe liver diseases within one year of age. Instead, some, but not all animals showed continued pro-inflammatory effects in the liver including infiltration and proliferation of CD45-positive leukocytes and hepatocytes. In addition, pathological assessment of liver histology revealed indications of extra-medullary hematopoiesis in livers of hgp130 expressing mice (Fig. 1D).

Altogether, our starting hypothesis could not be validated. Our data implicates that constitutive activation of the gp130 signalling pathway in the liver may result in slight chronic inflammation, continuous liver cell proliferation and extra-medullary haematopoiesis. However, in our hands constitutive gp130 activation did not result in liver cancer or otherwise severe liver disease.

**Promoting of young researchers**

**Doctoral Theses**

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<tr>
<th>Name</th>
<th>Year</th>
<th>Institution</th>
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<tr>
<td>Maruta, Y.</td>
<td>2017</td>
<td>Showa University, Fujigaoka, Yokohama, Japan</td>
<td>Gp130 signalling and migration of podocytes</td>
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<td>Baack, C.</td>
<td>2017</td>
<td>RWTH Aachen, Faculty 10</td>
<td>IL-6 signalling in glomerular epithelial cells</td>
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Do CREM/CREB mediated pathways regulate pro and anti-inflammatory properties of macrophages?

Tenbrock, K. (Department of Child- and Adolescent Medicine)

Current paradigms suggest that pro-inflammatory M1 macrophages enhance while anti-inflammatory M2 cells suppress inflammation. The cAMP responsive element modulator (CREM) belongs to the bzip family of transcription factors, which include the transcriptional activator CREB. We identified a role for CREM and CREB in NLRP3 inflammasome activation and showed that a pharmacologic inhibitor of the cAMP pathway is able to inhibit the NLRP3 inflammasome in a Caspase 1 dependent manner resulting in decreased IL-1ß production. CREM and CREB deficient macrophages therefore both show decreased IL-1ß production, which suggests proinflammatory properties of both transcription factors in macrophages.

Several models have been tested which test this inhibitor, including an atherosclerosis model, which showed effectiveness of propranolol. In addition, in vitro analyses to investigate the mechanisms how CREM and CREB regulate inflammasome activation are on their way.

In addition, a genetic deletion of CREB showed enhanced expression of IL-10 in macrophages. This was corroborated in a model with a Foxp3 specific deletion of CREB, which also resulted in enhanced suppressive function of regulatory T cells in several models including experimental transfer colitis and experimental autoimmune encephalitis. Results of these models have been used to ask for funding by DFG.

Applied third-party funding (DFG, BMBF, EU, foundations)

| Trautwein, C. | LiSyM Pillar II BMBF-Verbundförderung | BMBF | 01/2016-12/2021 | € 980,000 |
| Trautwein, C./ Hatting, M./ Liedtke, C./ Nevzorova, Y. | Application for a Collaborative Research Center: Gut-Liver axis – Functional Circuits and Therapeutic Targets | DFG/SFB1382, Projects A2 and A9 | concept paper submitted; pending result |

Promoting of young researchers

| Woltok, M. | Ongoing | RWTH Aachen, Faculty 1 | Modification of inflammation-triggered pathways leading to HCC development |

Funding period: 01.07.2014 - 31.12.2017
Staff: TV-L13 (50%): Ohl, K.
We examined our hypotheses that the progression from salutary pulmonary inflammation to organ damaging inflammation, which we term hyperinflammation, depends on the activation of a hyperinflammation module with specific mediators. Therefore, we characterized the modules inflammation and hyperinflammation in two ARDS models (acid- and LPS-induced lung injury) in ventilated mice by determining representative mediators and transcription factors. In the LPS model, we further analysed the kinetics of pro-inflammatory mediator expression by qRT-PCR and multiplex ELISA. These results serve as basis for driving inflammation into hyperinflammation by instilling identified hyperinflammation mediators, e.g. TNF, Cxcl10, IL-1α and LIF, intratracheally (i.t.) at the time-point of their liberation into mildly pre-inflamed lungs.

In addition, we investigated the lung-kidney crosstalk in collaboration with project Raffetseder (E7-7) in heterozygous YB-1 deficient (Yb1+/−) mice, which were protected from acid- and LPS-induced lung injury (Fig.1A). In these models, YB-1 was secreted into the alveoli (Fig.1B) and also upregulated in the kidneys. In 7h LPS-experiments, kidney injury was further indicated by increased Ngal expression (Fig.1C) and granulocyte infiltration. Additional ex vivo stimulation experiments in precision cut kidney slices revealed that acid as well as the damage-associated molecular pattern HMGB1, which was also found in injured lungs, induce YB-1 secretion (Fig.1D). The relevance of HMGB1 in hyperinflammation was also reflected by in vivo experiments in which an i.t. administered anti-HMGB1 antibody protected mice completely from acute lung injury. Finally, i.t. application of recombinant YB-1 into healthy mice demonstrated that extracellular YB-1 causes tubular kidney injury (Fig.1E), but does not harm the lung. Taken together, we suggest YB-1 as potential transmitter of secondary kidney injury in ARDS.
Lipocalin 2 (LCN2), a central mediator in inflammatory organ disease

Weiskirchen, R.
(Institute of Molecular Pathobiochemistry, Experimental Gene Therapy and Clinical Chemistry)

Borkham-Kamphorst, E.
(Institute of Molecular Pathobiochemistry, Experimental Gene Therapy and Clinical Chemistry)

In our previous work we showed that Lipocalin 2 (LCN2) is strongly activated during hepatic inflammation and a critical regulator influencing mitochondrial and peroxisomal integrity by regulating pathways involved in fat metabolism. In a further step we investigated if LCN2 has impact on fructose-induced liver damage. Therefore, we fed wild type and Lcn2−/− mice for 4 or 8 weeks on diets enriched in fructose either by adding this sugar to the drinking water (30% w/v), or by feeding a chow containing 60% (w/w) fructose. Body weight and daily intake of food and water of these mice was measured. Fat content in liver sections was visualized using Oil Red O stain. The expression levels of genes involved in fat and sugar metabolism were measured by qRT-PCR and Western blot analysis. We found that fructose-induced steatosis and liver damage was more prominent in female than in male mice. Moreover, the most severe hepatic damage occurred in female mice lacking LCN2 (Figure 1). Unexpectedly, consumption of elevated fructose did not induce de novo lipogenesis. We conclude that LCN2 acts in a lipid-independent manner to protect the liver against fructose-induced damage.
Publications

Peer-reviewed Originals resulted from the present IZKF project


Peer-reviewed editorials, reviews or book chapters resulted from the present IZKF project


Weskicrzen R (2017) Therapeutic targeting of the mitochondrial reactive oxygen species engine prevents portal hypertension and hepatic fibrogenesis. Liver Int 37, 963-965 [IF 4.116]


Applied third-party funding (DFG, BMBF, EU, foundations)

Weskicrzen, R. Targeting CTNN1/DYB1 as a new treatment modality in hepatic fibrosis DFG/BR 57 (P13) 01/09-12/20 € 1,200,000

Weskicrzen, R./Tacke, F. FACS-based cell isolation and clinical translation DFG/BR 57 (S03) 01/09-12/20 € 1,510,000

Weskicrzen, R. Scholarship for politically persecuted Philipp Schwartz-Initiative der Alexander von Humboldt-Stiftung mit Unterstützung des Auswärtigen Amtes (3. Runde) 08/17-07/19 € 90,000

Weskicrzen, R. Promotion of international research mobility between India and Germany DAAD 01/18-12/19 ~ € 20,000


Fricke, G./Weskicrzen, R./Stremmel, W. New therapeutic approaches for the treatment of Wilson disease DFG (Einzelauftrag) Submitted, pending result


Peer-reviewed publications resulted from cooperation with other IZKF projects of the consortium


**The role of YB-1 in reversible models of renal inflammation**

**Raffetseder, U. (Department of Internal Medicine II)**

The highly conserved cold-shock protein Y-box protein (YB-1) constitutes a major, non-redundant mediator in both, systemic and local inflammatory responses. Within our project, we could confirm a decisive role of YB-1 in the onset and resolution of acute and chronic inflammation in (reversible) renal inflammation models via regulation of the anti-inflammatory factor IL-10 (1).

In collaboration with project Borkham-Kamphorst/Weiskirchen (E7-6) we further revealed that Yb1+/− mice exert differences in induction and in the course of hepatic inflammation ( bile duct ligation (BDL) ) with impact on renal damage/inflammation. Two weeks following BDL surgery, the hepatic expression of extracellular matrix proteins (Fig. 1 A) and α-smooth muscle actin (Fig. 1 B) was significantly reduced in Yb1+/− compared to WT mice. Against this, the expression of chemokine CXCL1 was elevated in Yb1+/− mice (Fig. 1 C). By further analyses, we subsequently identified YB-1 as a potent transcriptional repressor of Cxcl1 gene expression (Fig. 1 D). Pharmacological inhibition of CXCL1 receptor CXCR2 by SB225002 significantly reduced early systemic effects on the kidneys following BDL (Figs. 1E/F). In contrast to liver, the systemic effects following BDL on kidneys were reinforced in Yb1+/− mice compared to their WT littermates. The tubular integrity, verified by the reactivity of Lotus tetragonolobus lectin (LTL) with the brush borders of proximal tubules (Fig. 1 G), the tubular damage, quantified in PAS-stained renal sections (Fig. 1 H), and the quantification of immune cell infiltration by CD45-positivity (Fig. 1 I) exhibited enhanced renal damage/inflammation in Yb1+/− compared to WT mice following BDL.

In summary, we demonstrated that YB-1 crucially contributes to hepatic and renal fibrosis/inflammation and identified Cxcl1 as well as IL-10 (1) as new target genes of YB-1. Furthermore, we identified YB-1 as an important modulator of organ crosstalk between liver/kidney and, in collaboration with Uhlig/Reiss (E7-5), also between lung/kidney.

**Publications**


Figure 1: In the BDL model in mice (14 days), half-maximal expression of YB-1 resulted in ameliorated hepatic fibrosis (A,B) but enhanced CXCL1 expression (C) as YB-1 represses the CXCL1 promoter (D). Pharmacological inhibition of CXCL1 receptor CXCR2 ameliorates early renal damage 4 days after BDL (E/F). Reinforced effects on renal damage (dilatation (d); ECM (*), necrosis (>)) and inflammation in Yb1

Tubular cells in acute kidney injury: repair or die.

Moeller, M. J. (Department of Internal Medicine II)

After the controversial results of in vitro experiments to assess the influence of the EGF signalling pathway on scattered tubular cells (STC) and non-STC, it was planned to perform transcriptomal analyses of the two cell types. We found that the previously established method to isolate STC and non-STC by fluorescence-activated cell sorting (FACS) did not provide living cells after the sort. The cells no longer adhered in culture any longer. Currently, the improvement of the FACS and problem solving methods are still in progress.

In March 2018, we plan to perform transcriptomal analyses of STC versus of non-STC, both either from healthy or ischaemia/reperfusion (IR)-injured mice. The FACS isolation is essential to isolate and separate the two cell types. In addition, single cell analyses of STC and non-STC will be established with the expertise of a collaboration partner at Monash University in Melbourne, for which external funding was granted by the DAAD.

During the funding period in the year 2016, the investigation of the effect of EGF receptor stimulation and inhibition, by administration of EGF or Erlotinib to mice, prior to or after IR injury induction, was performed. The necessary experimental groups of this study were completed with additional mice in 2017. Our results show that there seems to be no difference in the STC phenotype after inhibition of EGF receptor by Erlotinib in comparison to vehicle treated mice. As well, there were no significant differences in the amount of STC seen between mice that were treated prior to the IR injury and the ones treated after surgery (Figure 1A).

The treatment of the mice with EGF led to an increase of STC number when EGF was administered during regeneration phase after IRI (Figure 1B). Interestingly, the STC marker kidney injury molecule 1 (KIM-1) was expressed at a higher level when the EGF receptor was either blocked (by Erlotinib) or stimulated (by EGF) prior to the injury induction (Figure 1A’ vs. B’). Also, EGF treatment had an effect on the expression of Sox9, while it was increased when EGF was given during the regeneration phase but not by administration in a preconditioned fashion (Figure 1B’). In contrast, Sox9 expression did not seem to be affected by the inhibition of the EGF receptor (Figure 1A’). Marker expression analyses of KIM-1, Sox9, p65, c-Jun as well as of the proliferation and the amount of STC is almost completed.

Funding period: 01.07.2014 - 31.12.2017

Staff:
TV-L13 (35%): Leuchtle, K.

Applied third-party funding (DFG, BMBF, EU, foundations)

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<th>Funding Period</th>
<th>Funding Amount</th>
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<tbody>
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<td>Notch-3 and its soluble ligand in the pathogenesis of lupus nephritis</td>
<td>01.2016-12.2018</td>
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<td>DFG RA 740/9-1</td>
<td>The role of YB-1 in the early pathogenesis in murine ARDS</td>
<td>07/2016-07/2019</td>
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</tr>
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</table>

Promoting of young researchers

<table>
<thead>
<tr>
<th>Name</th>
<th>Year</th>
<th>Institution</th>
<th>Thesis Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Houben, N.</td>
<td>2017</td>
<td>RWTH Aachen, Faculty 1</td>
<td>YB-1 is involved in NET formation in granulocytes</td>
</tr>
</tbody>
</table>

Doctoral Theses

<table>
<thead>
<tr>
<th>Name</th>
<th>Ongoing</th>
<th>Institution</th>
<th>Preliminary Title</th>
</tr>
</thead>
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<tr>
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<td>Ongoing</td>
<td>RWTH Aachen, Faculty 1</td>
<td>The impact of Notch-3 and its soluble ligand YB-1 in chronic inflammation</td>
</tr>
</tbody>
</table>
Role of ADAM-family metalloproteinases for resolution and progression of lung and kidney inflammation

Ludwig, A. (Institute for Pharmacology and Toxicology)
Ostendorf, T. (Department of Internal Medicine II)

The “A disintegrin and metalloproteinase” (ADAM) family members ADAM8, 10 and 17 are involved in numerous shedding events implicated in proliferation, inflammation and fibrosis. Our project aims to identify cell type specific functions of ADAMs in experimental lung and kidney inflammation and fibrosis. Recently we showed that ADAM8 is implicated in the migratory response of neutrophils and monocytes and that ADAM8 deficiency in mice reduces alveolar recruitment of these cells in a model of LPS-induced acute lung inflammation. However, we did not observe an effect of ADAM8 knockout on bleomycin-induced lung fibrosis. This indicates that ADAM8 is implicated in acute inflammatory responses but not in development of fibrosis in the lung. A major focus of the last year was on the role of ADAM10 in kidney fibrosis. We used a model of unilateral ureteral obstruction (UUO) to generate interstitial renal fibrosis in mice. For this, the left ureter was ligated, leading to rapid hydronephrosis and within days to tubulointerstitial damage. The right ureter was not ligated and additionally sham-operated mice were used as controls. Using transgelin as a marker for smooth muscle cells and myofibroblast we observed considerable increase of transgelin positive cells including smooth muscle cells, parietal epithelial cells and interstitial cells in the cause of fibrosis development only in left kidneys with ligated ureter (Figure 1). To further investigate the role of ADAM10 mediated shedding events in these cells we decided to cross transgenic mice expressing cre recombinase under the control of the transgelin promoter with mice carrying floxed ADAM10 gene to generate ADAM10 knockout in transgelin positive cells. In initial experiments, we studied control animals that were homozygous for floxed ADAM10 and negative for cre recombinase as well as wild type animals that were negative for recombinase and carried both wildtype ADAM10 alleles. However, the simple presence of two floxed ADAM10 alleles lead to increased influx of macrophages, lymphocytes and neutrophils as well as increased fibrotic markers like α-smooth muscle actin, collagen 3 and fibronectin. These unforeseen results point out how important it is to use the appropriate control animals. For this reason we had to refine our animal experiment for which we received new approval recently. We now directly compare only litter mice which carry both floxed ADAM10 alleles either in the absence or presence of cre recombinase. The project will be continued and results are expected in 2018.

Figure 1: Transcriptional decision in acute kidney injury: effect of EGF receptor pathway on STC.

Applied third-party funding (DFG, BMBF, EU, foundations)

Figure 1: Upregulation of transgelin positive cells in fibrotic kidneys.

Publications


Applied third-party funding (DFG, BMBF, EU, foundations)

Ludwig, A. Role of ADAM family proteases in chronic lung inflammation

Promoting of young researchers

Doctoral Theses

Schumacher, J. Completed in 2018 RWTH Aachen, Faculty 10 Role of ADAM proteases in experimental lung fibrosis

Wozniak, J. Ongoing RWTH Aachen, Faculty 10 Role of ADAM proteases in kidney inflammation and fibrosis

Giese, A. Ongoing RWTH Aachen, Faculty 10 Inflammatory regulation of rhomboid pseudoproteases
### O1 JOINT RESEARCH PROJECT

**Brümmendorf/Koschmieder**
Consortium to study Myeloproliferative Neoplasms (MPN)

<table>
<thead>
<tr>
<th>Project</th>
<th>Authors</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>O1-1</td>
<td>W. Wagner/Jost</td>
<td>118</td>
</tr>
<tr>
<td>O1-2</td>
<td>Seré/Koschmieder</td>
<td>121</td>
</tr>
<tr>
<td>O1-3</td>
<td>Müller-Newen/Schemionek</td>
<td>124</td>
</tr>
<tr>
<td>O1-4</td>
<td>Zenke/Chatain</td>
<td>126</td>
</tr>
<tr>
<td>O1-5</td>
<td>Schneider-Kramann/Ziegler</td>
<td>128</td>
</tr>
<tr>
<td>O1-6</td>
<td>Brümmendorf/Schuppert</td>
<td>130</td>
</tr>
<tr>
<td>O1-7</td>
<td>Huber</td>
<td>132</td>
</tr>
</tbody>
</table>

### O2 JOINT RESEARCH PROJECT Lüdde
Defining Road Blocks against Liver Cancer

<table>
<thead>
<tr>
<th>Project</th>
<th>Authors</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>O2-1</td>
<td>Lüscher/Lüdde</td>
<td>135</td>
</tr>
<tr>
<td>O2-2</td>
<td>Lüdde</td>
<td>137</td>
</tr>
<tr>
<td>O2-3</td>
<td>Tacke</td>
<td>139</td>
</tr>
</tbody>
</table>

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**PROJECTS**

**Oncology**
Consortium to study Myeloproliferative Neoplasms (MPN)

Brümmendorf, T. H. and Koschmieder, S. (Department of Hematology, Oncology, Hemostaseology, and Stem Cell Transplantation)

Since its initiation in 2014, the Myeloproliferative Neoplasm (MPN) IZKF consortium has become a very active node of innovative and collaborative research at the Faculty of Medicine of RWTH Aachen University. This was accomplished through:

- active research by the research groups including the funded PhD students in the individual consortial projects (see individual final reports)
- continued monthly MPN progress meetings of up to 40 different researchers, including the consortial project leaders, and complemented by external speakers (e.g. Robert Kralovics from Vienna)
- joint publications by the project leaders (over 50 joint publications in 2014-2017)
- successful acquisition of DFG and other external funding in the field of MPN (3 DFG individual projects [Kramann/Schneider, Schemionek, Koschmieder] and 1 DFG research fellowship [Bayer/Schneider]) and inclusion of MPN as a dedicated clinical translational use case into the excellence cluster initiative application "predictive hierarchical clustering (PNH)"
- propagation of clinical excellence (specialized MPN clinic, next-generation sequencing-based personalized molecular diagnostics)
- conduct of prospective multi-center clinical investigator-initiated trials (Ruxo-BEAT and BODO led by Aachen and Aachen/Bonn, resp.) actively enrolling patients and acquiring biomaterial for translational science projects
- foundation of the German Study Group for MPN (GSG-MPN) and its associated bioregistry in 2015, which has recruited over 2,500 patients and over 13,000 biomaterial samples
- Organization and hosting of the first combined GSG-MPN Study Group Meeting and MPN/CML Scientific Meeting, which was held in Aachen in April 2017 and attracted 170 participating scientists and clinicians.

Overall, the IZKF MPN consortium has been a great success and will continue to demonstrate its positive effects in our Faculty, the Euregio region, and beyond.
Epimutations and Splice Variants of DNMT3A and TET2 in Myeloproliferative Neoplasms

Wagner, W.  
(Institute for Biomedical Engineering – Stem Cell Biology and Cellular Engineering)  
Jost, E. (Clinic for Oncology, Hematology and Stem Cell Transplantation)

The de novo DNA-methyltransferase DNMT3A is a key modulator of DNA methylation (DNAm). It is frequently mutated in myeloproliferative neoplasms (MPN) and acute myeloid leukemia (AML), and is alternatively spliced in a tissue- and disease-specific manner. So far, little is known about the functional roles of different DNMT3A variants. The objective of this project is to elucidate their specific function and role in pathogenesis of MPN and/or AML.

Individual transcripts of DNMT3A were knocked down (KD) by shRNA or overexpressed (OE) in cord blood-derived HPCs (Figure 1a). Altered expression was validated by qRT-PCR (Figure 1b; n=3). DNAm was analyzed with the Human Methylation 450K Chip and revealed significant and transcript-specific methylation changes (n=3; adjusted p-value<0.05; Figure 1c). In contrast, knockdown of Tr.4, which does not exhibit methyltransferase activity, did not reveal any significant changes (KD Tr.4, Figure 1c). Differentially methylated CpGs from the knockdown were significantly regulated in the opposite direction in the overexpression experiment, indicating that the transcript specific epigenetic modifications are indeed specific for different DNMT3A variants (Figure 1d). Analysis of global gene expression profiles with the Affymetrix chip technology revealed 46, 225, and 190 differentially expressed genes upon knockdown of Tr.1+3, Tr. 2, and Tr.4, respectively (n=3; adjusted p-value<0.05; 1.5 fold change; Figure 1e). Thus, there are also transcript-specific effects on gene expression signatures. Next, we examined the proliferation potential and immunophenotype (CD34) of each DNMT3A splice variant. Progenitors proliferated slower upon knockdown of Tr.2 and Tr.4 (n=3; p<0.05), and slightly faster upon their overexpression compared to the control (n=3; Figure 1f). Expression of CD34 was maintained even in the fast proliferating fraction when Tr.2 was downregulated (n=3; p<0.05), whereas overexpression reduced the proportion of CD34+ cells in this fraction (Figure 1g; n=3). The differentiation potential of individual transcripts was determined by the colony forming unit (CFU) assay. Notably, the number of erythroid colonies was increased when Tr.4 was downregulated (n=3; p<0.01; Figure 1h) and decreased upon overexpression (not shown). We further examined the in vitro transcript-specific DNAm and gene expression signatures in AML samples from the TCGA repository. In fact, the in vitro signatures of Tr.2 were coherently modified in AML (data not shown) and expression of this DNMT3A transcript correlated with overall survival (Kaplan-Meier P<0.019; Figure 1i; Cox regression P = 0.016).

Our results demonstrate that DNMT3A variants have transcript-specific effects on global DNAm and gene expression profiles and have unique regulatory functions during the differentiation process of blood progenitors. Moreover, signatures of Tr.2 are recapitulated in AML, and its expression in AML patients correlates with clinical parameters.

Funding period: 01.07.2014 - 31.12.2017

Staff:  
TV-L13 (50%): Božić, T.
IRF8 as Tumor Suppressor and Modulator of Interferon Response in Myeloproliferative Neoplasm (MPN) Stem/Progenitor Cells: critical implications for MPN pathogenesis and immunotherapy

Koschmieder, S. (Department of Oncology, Hematology and SCT)  
Seré, K. (Institute for Biomedical Engineering – Department of Cell Biology)  

We studied the molecular mechanism regulating the interferon-alpha (IFNα) response in MPNs such as chronic myeloid leukemia (CML) and polycythemia vera (PV). Loss of the transcription factor IFN regulatory factor 8 (IRF8) has been demonstrated to induce a CML phenotype. Here, we investigated how loss of IRF8 impacts on the response to tyrosine kinase inhibitor (TKI) and IFNα treatment. To do this, we overexpressed Bcr-Abl in IRF8+ and IRF8− mouse bone marrow (BM) cells and found that loss of IRF8 leads to increased cell expansion and less pronounced response to TKI, while the response to IFNα was maintained (Figure 1A). A more detailed analysis revealed that loss of IRF8 promoted expansion of mature Gr1+ cells and poor response to TKI in these cells, while the IFNα response was retained. Similar response profiles were seen in IRF8− immature ckit+ cells (Figure 1B).

Furthermore, we generated induced pluripotent stem cells (iPSC) from JAK2V617F+ patients to study IFNα response in PV stem cells (Figure 1C, left panel). Patient-derived JAK2WT and JAK2V617F iPSC efficiently differentiated into hematopoietic stem and progenitor cells (HSPC) (Figure 1C, right panel). Importantly, the JAK2V617F mutation enhanced erythropoietic differentiation from HSPC, thereby recapitulating the PV phenotype in vitro (Figure 1D). Next, we treated these iPSC-derived HSPC with IFNα. Unexpectedly, we found that JAK2WT and JAK2V617F HSPC responded poorly to IFNα in a viability assay and that they showed low upregulation of IFNα target genes compared to peripheral blood mononuclear cells (PBMC) (Figure 1E - G). One potential explanation might be low STAT1 and pSTAT1 protein levels (Figure 1H). Current investigations focus on further dissection of the underlying mechanisms in these PV stem cell counterparts.

In summary, we observed that IFNα can activate IFNα target genes in the presence of Bcr-Abl, and thus it will now be important to investigate whether this is also true in the absence of IRF8. Furthermore, we now have a human cellular model of PV in vitro using iPSC cells, and in order to understand how IFNα can eradicate JAK2V617F+ cells, it will be important to study if IRF8 has a role in the response of IFNα in PV. In addition, we will retrovirally transduce IRF8− and IRF8+ mouse marrow cells with murine JAK2V617F. These complementary approaches will allow us to study the effect of IRF8 in both CML and PV model systems.
Figure 1: IFNα responses in CML and PV (A and B) BcrAbl was overexpressed in IRF8+/+ and IRF8−/− mouse BM cells by retroviral transduction. Cells were deprived of growth factors for 2 days and then treated with TKI, IFNα or left untreated. Start of treatment was day 0. Every 24h, cells were quantified with an electronic cell counter and subjected to flow cytometry. (A) The number of BcrAbl+ cells is presented as normalized value to day 0 of treatment. (B) Gr1+ and ckit+ cell populations were analyzed by flow cytometry, and quantification of cell numbers after 4 days of treatment is shown. Data in A and B represent means ± standard deviation of three independent experiments. Statistical analyses were done with Mann-Whitney-U test by comparing TKI and IFNα treated to untreated cells. (C - H) Patient-derived JAK2WT and JAK2V617F iPSC were used to study IFNα response in PV. (C) Representative microscopic analysis of iPSC colonies (left panel) and flow cytometry analysis of CD31+CD43+HSPC on day 18 of hematopoietic differentiation is shown (right panel). (D) JAK2 and JAK2V617F HSPC were differentiated into red blood cells by addition of 0.2 U/ml EPO. After 12 days, cells were analyzed by flow cytometry and spun onto glass slides for histological staining with neutral benzidine/DiffQuik. Representative examples are shown. Arrows indicate normoblasts. (E - H) IFNα treatment of JAK2WT and JAK2V617F HSPC. (E) Cells were treated with varying concentrations of IFNα. After 48h, cell viability was measured using an MTT assay. Two independent experiments were performed in triplicates for each data point. Data points are means ± standard deviation. Puromycin (puro) treatment was used as control. (F) Two JAK2WT and JAK2V617F iPSC clones were differentiated into HSPC (n=4 for clone 1, n=3 for clone 2) and treated with 200 U/ml IFNα for 4h. RIN was isolated and subjected to RT-qPCR. (G) PV patient PBMC were thawed, healthy PBMC were isolated via density gradient centrifugation and treated and processed as cells in F (n=3). In H and G gene expression for IRF7 normalized to GADPH is shown. Statistical analysis was done using an unpaired Student’s t-test. (H) HSPC were deprived of growth factors for 4h, followed by a 2000U/ml IFNα pulse for 30min. SDS-Page and Immunoblotting were performed, and the membrane was probed with antibodies for STAT1, pSTAT1 and Actin. 293T cells transfected with human JAK2V617F were used as control (ctrl). For statistical analysis, p values ≤ 0.05 were considered significant (*), p-values ≤ 0.01 were highly significant (**).
STAT3-mediated Therapy Resistance of Malignant Stem Cells in Myeloproliferative Neoplasms (MPN)

Schemionek, M.
(Department of Hematology, Oncology, Hemostaseologie and SCT)
Müller-Newen, G. (Institute for Biochemistry and Molecular Biology)

Chronic myeloid leukaemia (CML) is currently treated with tyrosine kinase inhibitors (TKI), which are not sufficient to eradicate the disease-driving CML stem cells. Persistent activation of the transcription factor STAT3 has been observed in CML stem cells in presence of TKI, mediated by the BM microenvironment. The aim of this project was to elucidate the role of STAT3 in CML stem cell persistence and find crucial factors for STAT3 activation.

We found that TKI treatment even increases STAT3 activation in CML cells in presence of mesenchymal stromal cell (MSC)-derived conditioned medium (CM). The tyrosine kinase JAK1 was identified as main driver of persistent STAT3 activation in Bcr-Abl-positive cells. Combined inhibition of Bcr-Abl and JAK1 reduced persisting STAT3 activation, the number of leukemic colony forming units (CFU) and increased apoptosis in Bcr-Abl transgenic mice and samples from CML patients. Using transgenic Bcr-Abl mice we observed a decrease of long-term hematopoietic stem cells upon combinatory Bcr-Abl/JAK1 inhibitor treatment and secondary stem cell transplantation. Finally we identified IL-6 as STAT3-activating cytokine since application of an inhibitory IL-6 receptor fusion protein efficiently blocked MSC-mediated STAT3 activation.

Applied third-party funding (DFG, BMBF, EU, foundations)

Müller-Newen, G./Schemionek, M. Impact of the IL -6/Jak1/STAT3 signaling axis on MPN pathobiology and disease progression
Abteilung DFG Forschergruppe: Targeting the Malignant Network in Myeloproliferative Neoplasms
2018 - 2020 rejected

Promoting of young researchers

Doctoral Theses

Küpper, M. K. Ongoing RWTH Aachen, Faculty 1
STAT3-mediated Therapy Resistance of Malignant Stem Cells in Chronic myeloid leukaemia (CML)
Induced pluripotent stem cells (iPS cells) with KIT D816V mutation for modeling myeloproliferative neoplasms (MPN)

Zenke, M. (Institute for Biomedical Engineering - Cell Biology)  
Chatain, N. (Department of Hematology, Oncology, Hemostaseology and Stem Cell Transplantation)

Precision medicine (also referred to as personalized medicine) aims at tailoring medical therapy to the specific disease and needs of the individual patient. Frequently, genomic profiling by DNA sequencing for specific disease causing mutations, such as KIT D816V in myeloproliferative neoplasms (MPN), is used for diagnosis and decision on medical treatment. However, genomic profiles do not take into account tissue specific features and/or disease associated (and often unknown) mutations. Thus, even for the same disease and genomic profile medical treatment can fail due to patient specific differences. Here we used induced pluripotent stem cells (iPS cells) to generate disease and patient specific cells with KIT D816V mutation (and isogenic controls) for disease modeling and compound screening (Figure 1A).

The KIT D816V mutation leads to constitutive activation of the KIT receptor tyrosine kinase and confers resistance against the tyrosine kinase inhibitor Imatinib (Gleevec/Glivec). KIT D816V causes aggressive systemic mastocytosis (ASM) and finally mast cell leukemia (MCL) in affected individuals and KIT D816V patients are often left without effective therapy.

In this study, we generated iPS cells from ASM and MCL patients with KIT D816V mutation. KIT D816V iPS cells were induced to differentiate into KIT D816V hematopoietic progenitor cells and used for compound screening (Figure 1A and B). We demonstrate that compounds effectively silence constitutive KIT D816V signaling (Figure 1C). Thus, the collection of KIT D816V iPS cells generated in the study should pave the way towards identifying and testing novel drugs (and/or drug combinations) for treatment of ASM and MCL. Additionally, the KIT D816V animal model generated (Pelusi et al., 2017) will allow testing drugs in vivo.

**Publications**


**Applied and actual third-party funding (DFG, BMBF, EU, foundations)**

Zenze, M. StemCellFactory: Automatic production, expansion and differentiation of induced pluripotent stem cells (iPS cells)  
EU/Bio.NRW 11/2018-06/2020 € 1,013 Mio

Bolm, C./ Brümmendorf T. H./ Zenke, M.  
Multiple scale analysis of KIT D816V mutation in leukemia: Following up compound action in KIT signaling in cells, tissues and the organism  
DFG/ERS 09/2016-08/2017 € 34,000

**Promoting of young researchers**

Doctoral Theses

Förster, M. 2017 RWTH Aachen Faculty 1 Induced pluripotent stem cells with KIT D816V mutation for modeling myeloproliferative neoplasia
Identification of cellular and molecular identities contributing to myelofibrotic transformation of mesenchymal stroma cells in MPN

**Schneider-Kramann, R.**
(Department of Hematology and Oncology, Medizinische Klinik IV)

**Ziegler, P.** (Clinic of Occupational Medicine)

Bone marrow fibrosis (BMF) develops in various hematologic and non-hematologic conditions and is a central pathological feature and major diagnostic criterion of myelofibrosis (MF). The cellular origin of BMF remains elusive, and novel cell-targeted therapeutics are needed. Here, we demonstrate using genetic fate tracing in two murine models of BMF that Gli1+ mesenchymal stromal cells (MSC) are recruited from the endosteal and perivascular niche to become fibrosis driving myofibroblasts of the bone marrow. Genetic ablation of Gli1+ cells abolished BMF and rescued bone marrow failure. Pharmacologic targeting of Gli proteins with GANT61 inhibited Gli1+ cell expansion, myofibroblast differentiation and attenuated fibrosis severity. We further demonstrate that the same pathway is active in human BMF and that Gli1 expression in BMF significantly correlates with fibrosis severity. Because GANT61 treatment reduced the myofibroblastic phenotype of human MSC isolated from patients with BMF, targeting Gli proteins might be a novel therapeutic strategy in BMF.

**Publications**

**Awards**
Johann Georg Zimmermann Award for Excellence in Cancer Research 2017 , Hannover (to R. Schneider)

Artur Pappenheim Award 2017 (to R. Schneider)

Innovationspreis der Deutschen Hochschulmedizin (to R. Schneider)

<table>
<thead>
<tr>
<th>Applied third-party funding (DFG, BMBF, EU, foundations)</th>
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</thead>
<tbody>
<tr>
<td><strong>Schneider, R. K.</strong> Dissecting the cellular and molecular dynamics of bone marrow fibrosis for improved diagnostics and treatment</td>
</tr>
<tr>
<td><strong>Schneider, R. K.</strong> Targeting the cellular origin of myelofibrosis</td>
</tr>
</tbody>
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<th>Appointments to other universities</th>
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<tr>
<td><strong>Schneider, R. K.</strong> Erasmus Medical Center, University Hospital Rotterdam, NL</td>
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**Funding period:** 01.07.2014 - 31.12.2017

**Staff:** TV-L13 (50%): Ziegler, S.
Myeloproliferative neoplasms (MPN) are multi-phasic, chronic, clonal stem cell disorders frequently characterized by oncogenic mutations in JAK2, CALR or c-MPL. The identification of prognostic and/or predictive biomarkers allowing to improve modelling of disease progression (i.e. phase transition) in JAK2-driven MPN is important in order to improve treatment and outcome of affected patients. For this purpose, we performed gene expression arrays of CD34+ bone marrow and peripheral blood mononuclear cells (BM-MNCs/ PB-MNCs) derived from 10 patients with primary myelofibrosis (PMF), 5 patients with polycythaemia vera (PV; all JAK2V617F positive), 3 patients with post-PV or post-ET (secondary)MF and 4 healthy controls. Principal component analysis (PCA) showed clear differences in expression patterns between the different MPN subtypes as well as compared to healthy controls. The expression of CXCR4 (confirmatory result, proof-of-principle) and of new promising genes like TNFAIP3, ATF3 and AREG (the latter playing a role in CML) was downregulated in several MPN entities (Figure 1 A and B). Interestingly, rather distinct patterns of genes Characteristic for the different MPN subtypes could be shown as well.

For the computational part, we additionally used support vector machine classifier on the pre-processed data out of clinical MPN-SAL database, which contains information for clinical variables such as cell counts, patient’s history and mutation type of 574 patients in five categories of disease stages (tables 1, 2).

As the previous results show (Figure 1 C and D), PCA applied on a data set including gene expression data from patients in various MPN stages, MPN[GSE2647] reveals a signature for disease progression, starting from healthy control samples, passing through Essential Thrombocytemia (ET) and Polycythemia Vera (PV) toward Primary Myelofibrosis (PMF) samples. Since it is not a linear progression along main principle components, we tested other non-linear dimension reduction techniques such as t-SNE to extract such a progression trend as well, but with only limited improvements. In addition, we developed a Gaussian Mixture Model (GMM) to convert the nonlinear, multi-variate progression to a one-dimensional progression marker. The resulting marker could significantly separate control samples and PMF’s. On the other hand, ET and PV didn’t show significantly different values on the resulting biomarker (Figure 1E).

Since the biomarker achieved by GMM is solely based on one dataset, doing the same procedure on another dataset may lead to different results. To solve this problem, we established universal coordinate systems based on Physiospace method [Lenz, M. et al., PLoS ONE, 2013] and by using gene expression data of different Chronic Myeloid Leukemia (CML) phases from different cell types along normal hematopoietic lineage differentiation [GSE47927]. Setting up these universal coordinate systems, we mapped MPN dataset [GSE2647] along 2 different general mechanisms, including CML disease progression and normal hematopoietic lineage differentiation. Mapping the MPN dataset to coordinates corresponding to progression mechanisms of CML, we could detect a significantly different behavior of PMF from ET and PV. This result indicates that integrating heterogeneous sources of data can give more information about the progression of MPNs.

Table 1: number of patients in MPN-SAL dataset

<table>
<thead>
<tr>
<th>Essential Thrombocytemia</th>
<th>Polycythemia vera</th>
<th>Primary Myelofibrosis</th>
<th>Post-PV-Myelofibrosis</th>
<th>POST-ET-Myelofibrosis</th>
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<tr>
<td>108</td>
<td>207</td>
<td>136</td>
<td>23</td>
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Table 2: Variables of MPN-SAL data used in SVM and Principle component analysis

<table>
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<tr>
<th>Age</th>
<th>Leucocyte counts</th>
<th>Platelets counts</th>
<th>Haemoglobin level</th>
<th>Haematocrit</th>
<th>MCV (Mean Cell Volume)</th>
<th>Normoblast count</th>
<th>LDH (lactate dehydrogenase)</th>
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</table>

Promoting of young researchers

Doctoral Theses

<table>
<thead>
<tr>
<th>Baumeister, J.</th>
<th>Ongoing</th>
<th>RWTH Aachen Faculty 10</th>
<th>The role of hypoxia signaling pathways in establishment and maintenance of JAK2V617F positive MPN and genome-wide gene expression analysis of JAK2V617F target genes</th>
</tr>
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<td>Montazeri, M.</td>
<td>Ongoing</td>
<td>RWTH Aachen Faculty 10</td>
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</table>
Systemic mastocytosis (SM) is a clonal mast cell (MC) disease exhibiting various characteristics from indolent mastocytosis to aggressive mastocytosis and MC leukemia (MCL). MCL is a devastating disease with an average life expectancy of two months. Mutations in the receptor tyrosine kinase KIT (CD117) can be found in most cases of SM with KIT\textsuperscript{D816V} being the most prevalent and detectable in more than 80% of patients. Complicating therapies, the D816V mutation renders KIT resistant against many tyrosine kinase inhibitors. We have found in the human MCL cell line HMC-1.2, which expresses KIT\textsuperscript{D816V}, that the MAPK pathways containing the signalling elements MEK-ERK and TPL2-JNK are acting in a pro-proliferative and pro-survival manner. Moreover, they are linked by the MEK-ERK pathway suppressing TPL2 expression and consequently activation of JNK. Thus, inhibition of MEK by the pharmacological inhibitor PD03 resulted in upregulation of TPL2 expression and activation of JNK, which attenuated the cytostatic and cytotoxic effects of PD03. Hence, the combination of MEK and JNK inhibitors induced an effective apoptotic response in HMC-1.2 MCL cells. Overexpression and knock-out studies with respect to TPL2 to substantiate our findings are in statu nascendi. Our findings have the potential to establish a pharmacological treatment of TKI-resistant KIT\textsuperscript{D816V}-positive MCL.

**Figure 1:** Combined inhibition of the MAP kinases MEK and JNK causes cytostatic and cytotoxic effects in KIT\textsuperscript{D816V}-positive MCL cells.
Defining Road Blocks against Liver Cancer: Mechanisms linking Cell Death with inflammatory Carcinogenesis

Lüdde, T. (Department of Internal Medicine III)

In this collaborative grant proposal, several researchers grouped together to explore novel molecular mechanisms along the sequence towards liver cancer. The aim of the collaborative effort was to bundle the specific methodological expertise of each member along the sequence from cell death towards inflammation to cancer in order to enable a comprehensive approach for each subproject. The funding helped to establish strong and "real life" collaborations between the groups, as demonstrated by several common publications of all three group leaders in highly ranked journals during the funding period (e.g. Hepatology, Nature Communications, Cancer Cell etc.). Two of three subprojects successfully applied for external funding with the German Research Council (DFG). Moreover, this IZKF funded proposal was the prerequisite for a successful application for an ERC Consolidator Grant. Together, the IZKF funding of three projects resulted in the successful acquisition of approximately 3 Mio. € of external funding (DFG and ERC), which means that the internal funding was highly efficiently used for generating papers in the highest journals of cancer research and raising external funding. Additionally, two publications that arose from this grant initiative were awarded with the "Best Paper Award" from our Faculty (2014 and 2017) and were also awarded with important national awards (e.g. the research award of the "Arbeitsgemeinschaft Internistische Onkologie"). Finally, the fruitful interactions between the three group leaders have been consolidated in several new internal and external consortia (IZKF Initiative Oncology 2017, Joint Research Proposal "The Gut Liver Axis" (Project Leader: Prof. O. Pabst).

Control of cell proliferation, death and migration by the mono-ADPribosyltransferase ARTD10

Lüscher, B. (Institute of Biochemistry and Molecular Biology)
Lüdde, T. (Department of Internal Medicine III)

The hypothesis of this project was that ARTD10 affects liver development and possibly hepatocellular carcinoma (HCC). This was based on our finding that ARTD10, a mono-ADP-ribosyltransferase, interferes with the NF-κB signaling pathway (Verheugd et al., Nature Communications 2013). The NF-κB pathway is important for HCC development. We generated a mouse, in which we are able to induce an Artd10-specific shRNA in response to doxycycline (Dox). Indeed, Dox treatment induced the shRNA and resulted in a substantial knockdown of Artd10 mRNA in all tissues. The effect was particularly efficient in the liver with a 20-fold and larger knockdown. However, this had no effect on liver development over 18 months. Due to breeding problems of this mouse line, despite crossing in new animals that were bought and imported into our animal house, we were unable to test the effect of chemical carcinogenesis. At present the line has not bred for 5 months. The low breeding efficiency developed over time and because of the foreseeable problems, we developed an additional project, that was carried out by Laura in parallel to the mouse experiments. In this project we asked the question whether so-called macrodomains in different (+)ssRNA viruses are of functional importance. Macrodomains are intimately associated with ADP-ribosylation and we had identified cellular macrodomains as mono-ADP-ribosylhydrolases previously (Rosenthal, Feijs et al., Nature Structural and Molecular Biology 2013). We were able to demonstrate that these viral macrodomains possess hydrolase activity (Eckei, Krieg et al., Scientific Reports 2017). More recent findings demonstrate the functional relevance of the catalytic activity of the macrodomain of Chikungunya virus and Sindbis Virus. The figure shows that the Chikungunya macrodomain is capable of removing mono-ADP-ribosylation from ARTD10 in cells taken from Eckei, Krieg et al., Scientific Reports 2017).

Publications
Hepatic Alarmins: Mechanisms of Release and Role in Hepatic Inflammation and Carcinogenesis

Lüdde, T. (Department of Internal Medicine III)

The hypothesis of this project was that certain mediators (the so-called alarmins) released during specific hepatic cell death (apoptosis vs. necroptosis) lead to chronic inflammation and consequently to hepatocellular carcinoma (HCC). This hypothesis was based on our previous study where we showed that certain cell death pathways either induced or inhibited HCC development (Vucur et al. Cell Reports 2013). In the current study, we have analysed the hepatic role of receptor interacting protein kinase 1 (RIPK1), a multifunctional regulator of cell death triggered by tumor necrosis factor (TNF). RIPK1 is regulated by post-translational modifications (phosphorylation and ubiquitination) and mediates both apoptosis and necroptosis. We showed that RIPK1 is dispensable for normal liver homeostasis but withholds an additional TNF-dependent pro-survival function in liver cells. As such, RIPK1 acts as a scaffold to prevent the pro-oxidative degradation of the E3-ligase TNF-receptor-associated factor 2 (TRAF2) in a kinase-independent manner, thereby inhibiting Caspase-8 dependent apoptosis and the release of specific pro-inflammatory alarmins in the presence of NF-κB activation. We confirmed the functional relationship between RIPK1 and TRAF2 and generated mice with combined ablation of Ripk1 and Traf2 in liver parenchymal cells (LPC). Consequently, RIPK1/TRAF2 LPC-KO mice developed spontaneous LPC apoptosis. This spontaneous apoptosis initiated a sequence of alarmin-release, regeneration and inflammation that resulted in the spontaneous development of aggressive HCC development in older mice. Interestingly, histological examination of human HCCs with different etiologies revealed a significant survival benefit for those patients that expressed both RIPK1 and TRAF2 in their tumors. In contrast, low or undetectable tumor expression of both molecules showed the worst prognosis, which suggested that the new anti-carcinogenic checkpoint controlled by RIPK1 and TRAF2 in mouse livers might be of great relevance in human hepatocarcinogenesis.

Publications

Modulating hepatic iNKT cell accumulation and maturation for liver cancer prevention and treatment

Tacke, F. (Department of Internal Medicine III)

Hepatocellular carcinoma (HCC) commonly arises in chronically inflamed livers, but also provokes anti-tumor immune responses. This functional dichotomy of inflammation in liver cancer warrants some caution before introducing immune-targeted therapies against hepatic inflammation and fibrosis, because mechanisms of tumor surveillance in the inflamed liver could then be comprised. One of these fibrotic pathways is the CXCR6-CXCL16 mediated attraction of lymphocytes. CXCR6 is an important recruitment and maturation factor for lymphocytes, particularly hepatic NKT cells. Our IZKF project identified a yet unrecognized mechanism of CXCR6-dependent lymphocytes in suppressing hepatocarcinogenesis in vivo. We employed WT and CXCR6-deficient mice to two independent primary liver cancer models (diethylnitrosamine/DEN; conditional hepatocyte-specific NEMO-deletion). CXCR6 signaling suppresses DEN-induced liver cancer development (Figure 1), but not inflammation-driven liver cancer (NEMO model). In both models, CXCR6 was needed for CD4 T and NKT cell accumulation in the liver. Livers from CXCR6-deficient mice subjected to the DEN cancer model lack CD4 T/NKT characteristic cytokines by MALDI analyses, alongside impaired activation of (purely sorted) hepatic CD4 T and NKT cells during hepatocarcinogenesis (Figure 1). Adoptive transfer of CXCR6-competent sorted CD4 T or NKT cells revealed the CXCR6-dependent immune surveillance function of these cells to recognize and eliminate pre-cancerous senescent hepatocytes. Thus, we herein show that CXCR6 has a previously unrecognized function in tumor surveillance via NKT- and CD4+ T cell-dependent senescence control, indicating that novel anti-inflammatory or anti-fibrotic immune modulatory therapies in the liver need to be validated regarding effects on hepatocarcinogenesis.

Publications


Figure 1: Hepatic CD4 and NKT cells control liver cancer development. Left: Wildtype (wt) and Cxcr6−/− mice were subjected to the diethylnitrosamine (DEN) liver cancer model, revealing higher tumor load in Cxcr6−/− mice. Right: Tumor-bearing livers of Cxcr6−/− mice showed reduced hepatic CD4 T-cells and NKT cells, alongside reduced TNFα and IFNγ protein levels (MALDI analysis).

Applied third-party funding (DFG, BMBF, EU, foundations)

<table>
<thead>
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<th>Sponsor</th>
<th>Grant Number</th>
<th>Duration</th>
<th>Funding</th>
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<tr>
<td>Tacke, F. NKT cells and CXCR6 in hepatocellular carcinoma</td>
<td>DFG, Ta434/3-1</td>
<td>05/2015-12/2020</td>
<td>€ 510,000</td>
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<td>Tacke, F. I3-STM: Immunotherapie durch synthetische Antikörper beim metastasierten Mammakarzinom</td>
<td>Leitmarkt Agentur NRW, LS-1-1-027</td>
<td>10/2016-09/2019</td>
<td>€ 431,220</td>
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</tr>
</tbody>
</table>

Promoting of young researchers

Doctoral Theses

<table>
<thead>
<tr>
<th>Name</th>
<th>Year</th>
<th>Institution</th>
<th>Theme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Krenkel, O.</td>
<td>2017</td>
<td>RWTH Aachen, Faculty 1</td>
<td>Immune-mechanisms in acute liver failure and cancer</td>
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<td>Wehr, A.</td>
<td>2017</td>
<td>RWTH Aachen, Faculty 1</td>
<td>CXCR6 in hepatic inflammation, fibrosis and carcinogenesis</td>
</tr>
<tr>
<td>Frank, A.</td>
<td>Ongoing</td>
<td>RWTH Aachen, Faculty 1</td>
<td>Development of an in vitro liver biochip system</td>
</tr>
</tbody>
</table>
RESEARCH GROUPS

Costa Filho
Biostatistics
p. 144

Haiss
Clinical Neurosciences
p. 147

Liehn
Heart Attack Research Team
p. 150

Strnad
Inflammation and Consequences
p. 153
Bioinformatics

Gesteira Costa Filho, I. (Institute of Biomedical Engineering – Cell Biology)

Ivan G. Costa Filho was born on 20.07.1977 in Rio de Janeiro Brazil. He completed a B.Sc. (2001) and M.Sc. (2003) degrees in Computer Science at the Federal University of Pernambuco, Brazil. Latter, he joined the Max Planck Institute for Molecular Genetics, Berlin as a research assistant and completed his Dr. rer. nat. in Bioinformatics at the Free University Berlin (2008). Before moving to Aachen, he was an Assistant Professor in Bioinformatics at the Federal University of Pernambuco, Brazil. Currently, he has been appointed W2 Professor in Computational Genomics in 2017 at the RWTH Aachen Medical Faculty.

Use computational methods to understand and solve biomedical problems.

Type 2 diabetes (T2D) is an ideal disease model for proposal of personalized treatment solutions. Current treatment of T2D is based on a combination of lifestyle changes and pharmacological therapies. However, there are no specific guidelines for how to use available anti-diabetic drugs to target the underlying genetic traits. In a collaborative work with Anders Rosengren (University of Gothenburg, Sweden), we have analysed genome-wide data from T2D patients to find novel biomarkers and evaluate therapeutic approaches targeting these genes.

In a first study, we have proposed a methodology to find receptor modules dysregulated in T2D patients using co-expression networks (Fig. 1). We demonstrate that the receptor Par3 is associated with increase in insulin secretion. Moreover, antibodies blocking PAR3 counteracted the insulin secretion of pancreatic beta cells (Hanzelmann et. al 2016). Next, we have performed an integrative analysis of gene expression and open chromatin data on T2D patients. We observe binding of the transcription factor Sox5 in genes, which are down regulated in T2D patients and are associated to open chromatin in precursors of islet cells. This indicates an association of Sox5 with islet cell maturation. Moreover, treatment of a T2D diabetes mouse model with inhibitors of a chromatin remodelling factor could rescue Sox5 expression and restore normal insulin secretion (Axelsson et al. 2017).

Applied and actual third-party funding (DFG, BMBF, EU, foundations)

Costa, I. G.  DFG Sachbeihilfe 10/2017-9/2020  € 188,000

Promoting young Researchers

Doctoral Theses

Kuo, C.  Ongoing  RWTH Aachen Faculty 1  Long noncoding RNAs in Dendritic Cell Differentiation
Ticconi, F.  Ongoing  RWTH Aachen Faculty 1  Sparse models for analysis of clinical genomics data
Li, Z.  Ongoing  RWTH Aachen Faculty 1  Computational Methods for Single Cell Open Chromatin
Clinical Neurosciences

Hais, F. (Department of Neuropathology and Department of Ophthalmology)

Place/Date of birth: Antibes (France) 26.05.1977

2000 - 2002 Postdoctoral Research Fellow, Prof. Dr. Bruno Weber, Institute of Pharmacology and Toxicology, University of Zurich, Switzerland.

2002 – 2007 Dissertation with Prof. Dr. Cornelius Schwarz, Ph.D. in Neural and Behavioural Sciences, Graduate School of Neural & Behavioural Sciences, International Max Planck Research School, University of Tübingen.

2000 – 2002 M.A. in Neural and Behavioural Sciences, Graduate School of Neural & Behavioural Sciences International Max Planck Research School, University of Tübingen

The long-term goal of our research is to understand how neuronal networks in different parts of the brain interact during perception and how this functional connectivity is modified during learning and decision-making.

The research group was focusing on studying two aspects of a decision-making task. The first one was to correlate pupal responses with discrimination performance of rodents performing a vibrotactile two alternative forced choice task. Pupillometry, the measure of pupil size and reactivity, has been widely used to assess cognitive processes. As such, changes in pupil size have been shown to correlate with arousal, locomotion, cortical state and decision-making processes. Due to the extent of the processes reflected by the pupil, we investigated pupillary responses in the context of behavioral state and task performance by recording pupillary transients of mice performing a vibrotactile two-alternative forced choice task (2-AFC). Our findings show that task engagement is reflected in the pupil size prior to stimulus presentation, with the pupil being larger when mice are disengaged from task versus when actively engaged (Fig. 1.). In addition, in a state of active engagement, pupillary dilations are larger for correct responses compared to incorrect responses. This phenotype is not specific to reward attainment per se but rather to the task relevance of the stimulus when coupled with reward. Importantly, in a task variant involving working memory, we show that, though pupillary dilations during active engagement mainly follow the animals’ response time (RT), they also reflect animal decision prior to RT, with pupillary transients being higher for correct than error responses even when animal performance is close to chance level. Our results provide further evidence of how pupil diameter reflects cognitive processes in a task relevant context, elucidating how different elements of behavior are manifested by pupillometry. The second main focus was to develop new a variant of the behavioral task used in our laboratory and to study cortical responses in the primary somatosensory cortex to stimulus used in this task. Both humans and rodents can compare two simultaneously applied vibrotactile stimuli that are spatially separated. However, the ability to discriminate two proximate stimuli may be challenging as neighboring cortical representations have a strong overlap in the upper layers of the neocortex. We developed a vibrotactile discrimination task using the simultaneous stimulation of two neighboring whiskers in head-fixed mice. Stimulation of each whisker (C1 and D1)
was associated with a specific drinking spout which delivered a reward. Neural activity in upper layers of primary somatosensory cortex (S1) was monitored using wide-field calcium imaging (GCaMP6f). The two whiskers’ somatotopic representation concurrently spread over S1 and rose in amplitude with increasing stimulus frequency. Surprisingly, mice were able to discriminate frequencies even when the two cortical representations were strongly overlapping. We then further investigated sensory activity using two-photon calcium imaging (R-GECO) and mapped the selectivity of individual neurons to single whisker deflections. Consistently with wide-field results, responsive neurons spanned layer 2/3 over several neighboring barrels, with their density being higher over the principal barrel. Thus, concurrent representations of the two whiskers were intermingled in a “salt-and-pepper” organization. Furthermore, a subset of neurons was only modulated by simultaneous deflection of the two whiskers, with suppressive or additive effects. These neurons with multi-whisker receptive fields, may form the substrate for the capability of the animal to discriminate simultaneous neighboring inputs that lead to highly overlapping representations in the upper layers of S1.

Figure 1: Pupillary reactions of mice performing a vibratotactile 2-AFC task reflect task engagement and predict animal decision. (A) Schematic of a trial sequence for the 2-AFC task. (B) Example of a pupillary trace determined for one behavioral session. Pupillary trace shown in blue is the percent change in pupil size relative to the largest pupil detected per session. Gaps in the pupillary trace are trials omitted from analysis due to blinking. Insets: examples of pupil detection in different frames. (C) Percent change in pupil size relative to largest pupil diameter reflecting baseline pupil dilations for different animal responses. (D) Percent change in pupil size relative to baseline period, prior to stimulus onset, showing dilations for different animal responses. (For figures C, D: correct (green), error (red), misses (blue)). Yellow rectangle represents stimulus. (E) Percent change in pupil size relative to baseline period, prior to stimulus onset, showing dilations for correct (green) and error (red) responses in a 2 seconds delayed response 2-AFC task. Yellow rectangle represents stimulus; vertical black line represents water spout presentation. (F) Time from stimulus onset to largest pupil dilation correlates with the delay in response possibility for both correct (green) and error (red) responses; Kendall rank correlation, all shaded error bars represent 95CI for all figures N=3 mice.

Publications

Promoting young Researchers
Doctoral Theses

| Ganea, D. | Ongoing | RWTH Aachen, Faculty 10 | Thalamocortical adaptation and the influence of synchrony on cortical network activity and animal behavior. |
| Güther, M. | Ongoing | RWTH Aachen, Faculty 10 | Wide-field voltage and calcium imaging of cortical networks during decision making. |
| Gardères, P.-M. | Ongoing | RWTH Aachen, Faculty 10 | Neuronal basis of inter-hemispheric interactions during bilateral frequency discrimination. |
Heart Attack Research Team

Liehn, E.-A. (Institute for Molecular Cardiovascular Research)

Place and date of birth: Craiova (Romania), 30.09.1974


The study of chemokines in cardiac and vascular remodeling, in the context of atherosclerosis and myocardial infarction and finding new diagnostic and therapeutical strategies to improve the patient’s care in current clinical practice.

Monitoring the vascular regeneration after therapeutic intervention is an important clinical diagnostic tool which should be taken into consideration in the near future. Using molecular ultrasound imaging, we continue our studies to detect useful markers for clinical implementation. We found out that JAM-A is an ideal marker to detect endothelial dysfunction due to temporary blood flow variations in the main arteries (Curaj et al, Arterioscler Thromb Vasc Biol, 2017). Therefore, its imaging may facilitate the early detection of cardiovascular risk areas, and it enables the therapeutic prevention of their progression toward an irreversible pathological state. Moreover, combining medical imaging and biology with textile engineering, new concepts in sustaining tissue healing by modifying immune response can be developed (Moog et al, Angewandte Chemie International Edition, 2017; Gajjala et al, Hypertension, 2017).

We have also standardized some models of induced neointima formation (Curaj et al, J Vis Exp, 2017) and aortic pathology (Rowinska et al, J Vis. Ex, 2017) to be able to deepen into the arterial wall pathology mechanisms.


Staff:
TV-L13: Balabanu-Curaj, A.; Rusu, M.; Wu, Z.; Staudt, M;
TV-L9: Soltan, R.; Freund, A.

Publications


Inflammation and consequences

Strnad, P. (Department of Medicine III)

I was born on 12.04.1977 in Liberec, Czech Republic. I attended a bilingual Czech-German high school in Liberec and studied medicine in Mainz. After that, I spent 3.5 years as a postdoc at Stanford university (California). Since 2008, I am working as a research group leader and an Emmy-Noether fellow in Germany (first in Ulm, since 2012 in Aachen).

I am trying to improve our understanding of complex digestive diseases and their modifying factors.

Since the beginning of IZKF funding, our work focuses on the importance of iron metabolism in context of human disease. As a part of this topic, we demonstrated that increased iron load in hepcidin knockout mice leads to the development of chronic pancreatitis (Lunova et al., 2017). As an underlying mechanism, hepcidin knockout mice showed increased pancreatic oxidative stress, with elevated DNA damage, apoptosis and activated nuclear factor-kB (NF-kB) signalling. Hepcidin supplementation led to an improvement in general health status and to iron redistribution from acinar cells to macrophages. It also resulted in decreased NF-kB activation and reduced DNA damage (Lunova et al., 2017).

Given my long-term interest in proteotoxic stress, we generated transgenic animals overexpressing the classic stress-inducible chaperone heat shock protein (Hsp)72 in hepatocytes (Hsp72 mice). Primary hepatocytes from these animals were more resistant towards the isolation-induced stress and in three different hepatotoxic models (acute acetaminophen overdose, chronic feeding with methionine choline-deficient or 3,5-diethoxycarbonyl-1,4-dihydrocollidine-supplemented diet) displayed lower hepatic injury (Levada et al., in press; Figure 1). Hsp72 overexpressors had a lower amount of acetaminophen-protein adducts (Figure 1) and were protected from oxidative stress and stress-induced cell death. Hsp72-LAP mice/hepatocytes displayed significantly attenuated JNK activation. Overexpression of Hsp72 did not affect the development of steatosis nor the extent of protein aggregate formation (Levada et al., in press).

As a new topic related to chronic proteotoxic stress, we began to analyze liver involvement in patients with alpha1 antitrypsin deficiency (AATD). AATD arises due to a mutation in alpha1 antitrypsin (AAT) gene that results in AAT accumulation within the endoplasmic reticulum and leads to development of lung and liver disease in susceptible individuals. We focus on the clinically most relevant mutation termed as PiZ. The heterozygous carriage of this mutation is quite common (approx. 2% of the general population in Germany) and our work demonstrated that it predisposes to development of advanced liver fibrosis in presence of a second hit such as alcoholic or non-alcoholic steatohepatitis (Strnad et al., submitted). The carriage of a homozygous PiZ mutation is much less frequent (1:2000-1:4000 individuals), but results in development of significant liver fibrosis in approx. 23%
of the affected individuals even in absence of an additional liver disease (Hamesch et al., submitted). Because of this pioneering work, we were asked by the European Association for the Study of the Liver (EASL) to establish an Europe-wide registry of patients with AATD.

Figure 1. Hsp72 overexpression protects from acetaminophen (APAP)-induced liver injury. (A) Hematoxylin and Eosin (H&E) staining reveals the liver architecture of Hsp72-overexpressing (TG) and non-transgenic animals (WT) prior to (Non-tr) as well as 4 and 18 hours after the treatment with APAP. Scale bar = 100 μm. (B) Serum levels of the liver injury marker alanine transaminase (ALT) and APAP serum levels (C) are shown as boxplots that indicate median with first and third quartile, while whiskers display smallest and largest non-outlier observations. (D) The amount of APAP protein adducts was assessed in the liver homogenates and is expressed as mean ± SEM.

Publications


Awards

Hamesch, K. Rotationssstipendium German liver foundation
Hamesch, K. Poster of Distinction, AASLD Liver Meeting® 2017
Hamesch, K. DAAD Reisestipendium, AASLD Liver Meeting® 2017
Hamesch, K. Hermann Strauß Abstract Preis, DGVS Annual Meeting 2017
Hamesch, K. Studiensiegel für klin. Studie zum Alpha1-Antitrypsin-Mangel, GVS
Heimes, C. Presidential Poster of Distinction, AASLD Liver Meeting® 2017
Kuscuoglu, D. Young Investigator Bursary, European Association for the Study of the Liver (EASL) 52th Annual meeting, 2017
Kuscuoglu, D. The Best Poster Award, Metabolism and Transport section, GASL German Association for the study of Liver, Essen, Germany 2017
Applied and actual third-party funding (DFG, BMBF, EU, foundations)

<table>
<thead>
<tr>
<th>Name</th>
<th>Project Title</th>
<th>Funding Body/Project</th>
<th>Duration</th>
<th>Funding (€/year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strnad, P.</td>
<td>Consequences of desmoglein 2 loss for organization and function of intestinal epithelial junctions</td>
<td>DFG- SPP 1782</td>
<td>4/2015-3/2018</td>
<td>~70T/year</td>
</tr>
<tr>
<td>Strnad, P.</td>
<td>Iron and α1-antitrypsin accumulation as hepatocellular initiators of liver fibrosis development</td>
<td>DFG- SFB TRR57 project n928</td>
<td>1/2017-12/2020</td>
<td>~110T/year</td>
</tr>
<tr>
<td>Strnad, P.</td>
<td>Epithelial factors contributing to development of digestive diseases</td>
<td>Else Kröner-Exzellentenzstipendium</td>
<td>1/2017-12/2018</td>
<td>~150T/year</td>
</tr>
<tr>
<td>Strnad, P.</td>
<td>The role of keratins in the liver.</td>
<td>DFG funding STR 1095/4-2</td>
<td>4/2017-3/2020</td>
<td>~90T/year</td>
</tr>
<tr>
<td>Strnad, P.</td>
<td>Modifiers of α1-antitrypsin deficiency-related liver disease</td>
<td>Alpha1 foundation</td>
<td>Applied</td>
<td>rejected</td>
</tr>
<tr>
<td>Strnad, P.</td>
<td>REGISTRY ON ALPHA1-ANTITRYPSIN DEFICIENCY-RELATED LIVER DISEASE</td>
<td>EASL</td>
<td>5/2017-4/2022</td>
<td>~20T/year</td>
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<tr>
<td>Strnad, P.</td>
<td>The role of α1-antitrypsin augmentation therapy on development of liver disease in patients with severe α1-antitrypsin deficiency</td>
<td>Grifols</td>
<td>1/2017-12/2019</td>
<td>~30T/year</td>
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</table>

Promoting young Researchers

Doctoral Theses

<table>
<thead>
<tr>
<th>Name</th>
<th>Year</th>
<th>Institution, Faculty</th>
<th>Thesis Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kuscuoglu, D.</td>
<td>2017</td>
<td>RWTH Aachen, Faculty 10</td>
<td>The role of iron metabolism in liver disease development</td>
</tr>
<tr>
<td>Mo, F.</td>
<td>2017</td>
<td>RWTH Aachen, Faculty 10</td>
<td>The mechanisms of liver injury due to α1-Antitrypsin mutation</td>
</tr>
<tr>
<td>Nuraldeen, R.</td>
<td>2017</td>
<td>University of Ulm, Medical Faculty</td>
<td>Hepcidin and Iron Parameters Determine Disease Outcome</td>
</tr>
<tr>
<td>Gonzior, C.</td>
<td>2017</td>
<td>University of Ulm, Medical Faculty</td>
<td>Untersuchung der protektiven Rolle des Hitzeschockproteins 72 bei akuter Pankreatitis</td>
</tr>
<tr>
<td>Core Facility</td>
<td>Page</td>
<td></td>
<td></td>
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<tr>
<td>---------------------------------------------</td>
<td>------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Using Institutes and Clinics</td>
<td>160</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Core Laboratory</td>
<td>162</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genomics Facility</td>
<td>165</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immunohistochemistry Facility</td>
<td>171</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Confocal Microscopy Facility</td>
<td>173</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain Imaging Facility</td>
<td>176</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Two-Photon Imaging Facility</td>
<td>184</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transgenic Service</td>
<td>189</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proteomics Facility</td>
<td>194</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flow Cytometry Facility</td>
<td>198</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Using Institutes and Clinics

<table>
<thead>
<tr>
<th>Clinical Departments</th>
<th>Core Facilities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Department of Cardiology, Pneumology, Angiology and Internal Medicine Intensive Care (Internal Medicine I)</td>
<td>Immunohistochemistry Facility, Confocal Microscopy Facility, Transgenic Service, Genomics Facility</td>
</tr>
<tr>
<td>Department of Cardiac and Thoracic Surgery</td>
<td>Genomics Facility</td>
</tr>
<tr>
<td>Department of Child and Adolescent Psychiatry and Psychotherapy</td>
<td>Brain Imaging Facility</td>
</tr>
<tr>
<td>Department of Dental Preservation</td>
<td>Confocal Microscopy Facility, Brain Imaging Facility, Two-Photon-Imaging-Facility</td>
</tr>
<tr>
<td>Department of Dermatology</td>
<td>Transgenic Service, Genomics Facility</td>
</tr>
<tr>
<td>Department of Diagnostic and Interventional Neuroradiology</td>
<td>Brain Imaging Facility</td>
</tr>
<tr>
<td>Department of Gynaecology and Obstetrics</td>
<td>Genomics Facility</td>
</tr>
<tr>
<td>Department of Neurosurgery</td>
<td>Immunohistochemistry Facility, Brain Imaging Facility</td>
</tr>
<tr>
<td>Department of Neurology</td>
<td>Brain Imaging Facility, Transgenic Service, Proteomics Facility, Genomics Facility</td>
</tr>
<tr>
<td>Department of Nuclear Medicine</td>
<td>Brain Imaging Facility</td>
</tr>
<tr>
<td>Department of Ophthalmology</td>
<td>Immunohistochemistry Facility, Confocal Microscopy Facility</td>
</tr>
<tr>
<td>Department of Orthopaedics and Trauma Surgery, main focus on orthopaedics</td>
<td>Immunohistochemistry Facility, Genomics Facility</td>
</tr>
<tr>
<td>Department of Otolaryngology and Plastic Head and Neck Surgery</td>
<td>Immunohistochemistry Facility, Two-Photon-Imaging-Facility</td>
</tr>
<tr>
<td>Department of Paediatric and Adolescent Medicine</td>
<td>Two-Photon-Imaging-Facility, Transgenic Service</td>
</tr>
<tr>
<td>Department of Plastic, Hand and Burns Surgery</td>
<td>Immunohistochemistry Facility</td>
</tr>
<tr>
<td>Department of Psychiatry, Psychotherapy and Psychosomatics</td>
<td>Brain Imaging Facility</td>
</tr>
<tr>
<td>Department of Oral Maxillofacial and Plastic Facial Surgery</td>
<td>Immunohistochemistry Facility</td>
</tr>
<tr>
<td>Department of Surgery</td>
<td>Immunohistochemistry Facility, Proteomics Facility, Genomics Facility</td>
</tr>
<tr>
<td>Department of Urology</td>
<td>Immunohistochemistry Facility</td>
</tr>
<tr>
<td>Internal Medicine II (Nephrology and Clinical Immunology)</td>
<td>Immunohistochemistry Facility, Confocal Microscopy Facility, Two-Photon-Imaging-Facility, Transgenic Service, Genomics Facility</td>
</tr>
<tr>
<td>Internal Medicine III (Gastroenterology and Metabolic Disorders)</td>
<td>Immunohistochemistry Facility, Confocal Microscopy Facility, Two-Photon-Imaging-Facility, Transgenic Service, Proteomics Facility, Genomics Facility</td>
</tr>
<tr>
<td>Internal Medicine IV (Haematology and Oncology)</td>
<td>Immunohistochemistry Facility, Confocal Microscopy Facility, Transgenic Service, Proteomics Facility, Genomics Facility</td>
</tr>
<tr>
<td>Institutes</td>
<td></td>
</tr>
<tr>
<td>Institute of Biochemistry and Molecular Biology</td>
<td>Confocal Microscopy Facility, Two-Photon-Imaging-Facility, Transgenic Service, Proteomics Facility, Genomics Facility</td>
</tr>
<tr>
<td>Institute of Biochemistry and Molecular Cell Biology</td>
<td>Confocal Microscopy Facility</td>
</tr>
<tr>
<td>Institute of Biochemistry and Molecular Immunology</td>
<td>Immunohistochemistry Facility, Confocal Microscopy Facility, Proteomics Facility, Genomics Facility</td>
</tr>
</tbody>
</table>

### External Cooparations

- DAW – Leibniz Institute for Interactive Materials
- Immunohistochemistry Facility

### Helmholtz-Institute for Biomedical Engineering (Applied Medical Engineering)

- Two-Photon-Imaging-Facility

### Teaching and Research Area Cardiovascular Engineering

- Confocal Microscopy Facility

### Teaching and Research Area Tissue Engineering and Textile Implants

- Genomics Facility

### Helmholtz-Institute for Biomedical Engineering (Experimental Molecular Imaging)

- Two-Photon-Imaging-Facility

### Helmholtz-Institute for Biomedical Engineering (Cell Biology)

- Two-Photon-Imaging-Facility, Transgenic Service

### Teaching and Research Area Stem Cell Biology

- Confocal Microscopy Facility, Genomics Facility

### Helmholtz-Institute for Biomedical Engineering (Biointerface)

- Immunohistochemistry Facility, Confocal Microscopy Facility, Transgenic Service, Proteomics Facility, Genomics Facility

### Institute of Clinical Chemistry and Pathobiochemistry

- Immunohistochemistry Facility

### Teaching and Research Area Molecular Pathobiochemistry and Experimental Gene Therapy

- Immunohistochemistry Facility, Confocal Microscopy Facility, Transgenic Service, Genomics Facility

### Institute of Human Genetics

- Immunohistochemistry Facility, Confocal Microscopy Facility, Transgenic Service, Genomics Facility

### Institute of Immunology

- Immunohistochemistry Facility

### Institute of Laboratory Animal Science

- Immunohistochemistry Facility, Two-Photon-Imaging-Facility, Transgenic Service

### Institute of Medical Psychology und Medical Sociology

- Brain Imaging Facility

### Institute of Molecular Medicine

- Genomics Facility

### Institute of Medical Microbiology

- Immunohistochemistry Facility, Transgenic Service

### Institute of Molecular and Cellular Anatomy

- Transgenic Service

### Institute for Molecular Cardiovascular Research

- Immunohistochemistry Facility, Confocal Microscopy Facility, Two-Photon-Imaging-Facility

### Institute of Neuromanyody

- Genomics Facility

### Institute of Neuropathology

- Brain Imaging Facility

### Institute of Neurosurgery

- Transitional Cell Research Facility, Proteomics Facility, Genomics Facility

### Institute of Occupational and Social Medicine

- Confocal Microscopy Facility, Proteomics Facility, Genomics Facility

### Institute of Pharmacology and Toxicology

- Two-Photon-Imaging-Facility, Transgenic Service, Genomics Facility

### Institute of Pathology

- Transgenic Service, Proteomics Facility, Genomics Facility

### Institute of Physiology

- Proteomics Facility, Genomics Facility

### Institute of Plant Cell Biology

- Proteomics Facility, Genomics Facility

### Institute of Human Genetics

- Proteomics Facility, Genomics Facility

### Institute of Human Genetics

- Proteomics Facility, Genomics Facility

### Institute of Human Genetics

- Proteomics Facility, Genomics Facility
Core Laboratory

Head of the Core Laboratory:
Liehn, E.-A.

Safety delegate / assistant project supervisor for genetic engineering security:
Preisinger, C.

Project supervisor for genetic engineering security:
Denecke, B.

Services
In the Core Laboratory various equipment is provided, that is not correlated to any Core Facility. Like the Core Facilities, the equipment is not only available for IZKF project heads or project personnel, but for all the members of the Faculty of Medicine. The equipment also can be used upon request by RWTH scientists. The Core Laboratory provides infrastructure, lab space and know-how. The personnel of the Core Laboratory provide services for research, assistance to use the equipment and advises in all scientific issues. The concept aims at handling organizational and technical-methodical challenges and assisting the project personnel. Furthermore the centralization of equipment and expertise aims at conserving resources. Synergetic effects emerge from the collaboration of researchers and Core Facilities and these effects are useful for the two-way technical-methodical assistance that keeps research and service at the highest level.

Users of the Core Laboratory
In 2017 the Core Laboratory was used by 263 employees of 31 institutes and clinics.

Equipment and contact persons
All equipment and laboratories can be used upon request and after having consulted the IZKF administration office and the responsible contact person.

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Contact Person</th>
<th>Contact Person</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopes</td>
<td>Wülfel, J./ Ensslen S.</td>
<td></td>
</tr>
<tr>
<td>Western Blot Documentation System LAS 3000</td>
<td>Preisinger C.</td>
<td></td>
</tr>
<tr>
<td>Flow Cytometry: FACS Calibur/FACS Canto</td>
<td>Tappe M.</td>
<td></td>
</tr>
<tr>
<td>Agilent Bioanalyser 2100</td>
<td>Kratz B.</td>
<td></td>
</tr>
<tr>
<td>7300 Real Time PCR Tax Man</td>
<td>Denecke B./ Kratz B.</td>
<td></td>
</tr>
<tr>
<td>Fluorostar Optima</td>
<td>Kratz B.</td>
<td></td>
</tr>
<tr>
<td>Nanodrop</td>
<td>Kratz B.</td>
<td></td>
</tr>
<tr>
<td>Photometer</td>
<td>Tappe M.</td>
<td></td>
</tr>
<tr>
<td>HPLC/FPLC</td>
<td>Preisinger C./ S. Gostek</td>
<td></td>
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<tr>
<td>MALDI-TOF System</td>
<td>Preisinger C./ S. Gostek</td>
<td></td>
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<tr>
<td>Geldoc – DNA and Protein</td>
<td>Ensslen S.</td>
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<tr>
<td>Autoclaves</td>
<td>Tappe M.</td>
<td></td>
</tr>
<tr>
<td>MilliQ Equipment</td>
<td>Kratz B.</td>
<td></td>
</tr>
<tr>
<td>Cell Culture Laboratory</td>
<td>Kratz B.</td>
<td></td>
</tr>
<tr>
<td>Constant Temperature Laboratory</td>
<td>Denecke B.</td>
<td></td>
</tr>
</tbody>
</table>
### Usage of the equipment

Following overview shows a selection of the equipment of the Core Laboratory and their usage in 2017:

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Using Clinics/Institutes</th>
<th>Total appointments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopes</td>
<td>19</td>
<td>312</td>
</tr>
<tr>
<td>LAS3000 (Western Blot)</td>
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<tr>
<td>FACS Canto</td>
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<td>465</td>
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<tr>
<td>Agilent Bioanalyzer</td>
<td>5</td>
<td>45</td>
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<tr>
<td>Nanodrop</td>
<td>15</td>
<td>910</td>
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<tr>
<td>Cell Culture Laboratory</td>
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<td>359</td>
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<tr>
<td>Taq Man</td>
<td>8</td>
<td>331</td>
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<tr>
<td>Fluorostar</td>
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<tr>
<td>Autoclaves</td>
<td>15</td>
<td>357</td>
</tr>
<tr>
<td>Light Cycler</td>
<td>1</td>
<td>47</td>
</tr>
</tbody>
</table>

### Skills/Services/Training courses/Consulting service

Our highly skilled team, well-equipped lab and computational infrastructure facilitate researchers access to the promising sequencing technologies (currently NGS) and classical Microarray technologies, enabling them to achieve their scientific goals more efficiently. Our current support includes all areas of molecular biological research with a focus on genome-wide analyses. This include (among others):

1. Technical and experimental consulting, especially implementation possibilities and limits of NGS-/Arrays techniques, options of data interpretation, publication of data, etc.,
2. Sample preparation from cell culture, tissue, and blood; or in accordance with favorite standard procedures (total RNA, miRNA, mRNA, DNA, protein) and RT-qPCR based experiments. This enables also scientists not familiar with molecular biological methods to implement NGS and/or array experiments in their projects.
3. Defined DNA fragmentation (Covaris Technology).
4. Quality control and quantification: analyses of DNA, RNA, and protein using the 2100 Bioanalyzer/Tape Station 4200 microfluidics-based platforms (Agilent); quantification using the Nanodrop and Quanitus (Promega).
5. Amplification, fragmentation and labelling technologies for array applications (source, material quantity, array and detection specific).
6. DNA/RNA sample preparation for NGS - LA. Library prep.
7. Array Hybridization.
8. NGS-Sequencing using the NextSeq500 (or MiSeq, if necessary)
10. Basic data analysis workflows for various NGS applications.
11. Bioinformatics Consulting
12. Advanced bioinformatics support tailored to project specific needs.
13. Development of analysis pipelines of emerging applications on request.
14. Easy access to analysis results or online services through our web interface
15. (time limited) storage of sequence data, recording of NGS- and Array-experiments and submission of results to GEO (Gene expression omnibus)
Core Facilities | Genomics Facility

Trainings and seminars in 2017:

Co-Organized:

- Tag der Medizinischen Forschung, 19 December 2017, Aachen, Germany
- NGS User Meeting: every second Wednesday of the month (UK Aachen - library of the Institute of Human Genetics):
- Presentation of the current services of bioinformatics in the Genomics Facility (12. July 2017)

Absolved:

- Attending the 9th International meeting 2017 Stem Cell Network NRW 16-18, May 2017 Muertzelt
- Bioinformatics workshop of the above mentioned meeting.
- 5th Annual GSCN Conference (11-13, September, 2017, Jena)
- EMBL course "Introduction to Next Generation Sequencing" 9-12, Oct. 2017 Genome Campus EMBL-EBI Cambridge United Kingdom.
- Practical Course in Bioinformatics/Software lab 2017 (Main topic: Bioinformatics for the nanopore technology), Institute of Computational Biology, Aachen.
- Webinar series of the Big Data to Knowledge programm of the National Institutes of Health (NIH).

Status and Development

The Genomics facility offers the implementation of most recent developments for NGS- and microarray-technologies and works continuously on adopting new trends at an early stage. Our goal is to improve all stages of genetic or genomic research projects. Users from a large number of institutes and clinics are using various microarrays applications at stable rates without significant changes over the current year. Additionally, the Genomics facility now established a variety of NGS applications. The number of NGS projects increased significantly compared to the last quarter of 2016, where we supported just few projects. By the end of June we started an NGS campaign to familiarise researchers with NGS and promote this technology within the medical faculty of RWTH Aachen. The campaign was quite successful as it gradually increased the variety of the user landscape, the usage rates of the NextSeq 500 sequencing machine and the number of applications supported by the Genomics facility. More specifically, we are now supporting the single cell 3’ solution of 10x Genomics at lab and bioinformatics level, allowing researchers access to the most promising single cell analysis. Furthermore, new specific NGS protocols, like CaptureSeq, are now part of our support landscape. At the end of January, Beate Kratz, a colleague with long experience, took a well-deserved retirement. At this point, we would like to take the opportunity of thanking Beate for her excellent performance and dedication over the last years. At the same time, we are happy to found a successor in Mélanie Mitchell. To meet new challenges we started, inter alia, working on the extension of our bioinformatics infrastructure. In the next year we will therefore have a substantial increase in our computational capacities, allowing for improved support and shorter waiting time. As in the previous years, the Genomics Facility offered advanced support to a large number of research projects, now also based on the NGS technology.
Users within the faculty
25 users from 17 institutes and clinics of the medical faculty uses the Genomics Facility.
The main users are Department of Internal Medicine III, the Institute of Pathology, the
Institutes of Biochemistry and the Institute of Human Genetics.

External users

Hollert H., Di Paolo C., Nüsser L. RWTH Aachen, Institute for Environmental Research (Biology V)
Kurts, C./Ludwig-Portugall, I./Liene, S. Institut für Experimentelle Immunologie, Universität Bonn (AöR)
Nattermann, J./Krämer, B. Ruprechts-Friedrich-Universität Bonn
Usadel, B. RWTH Aachen, Institute of Biology I

Collaborations
The Genomics Facility has project collaborations with the CyTuvax BV - Basic Pharma
BV, the Natural Science and Engineering Research Council of Canada (NSERC), the
Institute of Complex Systems 7: Biomechanics, Forschungszentrum Jülich, the Clinic for
plastic Surgery at the universityhospital Bergmannsheil in Bochum, the Stem Cell Biology
and Regenerative Medicine School of Pharmacy, University of Reading, United Kingdom,
the Faculty of Health - Department of Medicine - Institute of Immunology & Experimental
Oncology - Center for Biomedical Education and Research (ZBAF) - AG Stem Cells -
Witten/Herdecke University, the Anatomische Anstalt, Lehrstuhl II – Neuroanatomie,
München and the Cellmorphology and Molecular Neurobiology - Faculty of Biology and
Biotechnology - Ruhr University Bochum.

Publications
Kontry, U. (2018) accepted for publication in Oncotarget [IF 2016 5.168]
Borosch, S.; Dahmen, E.; Beckers, C.; Stoppe, C.; Buhr, E.M.; Denecke, B.; Goetzenich,
A.; Kraemer, S. (2017) Characterization of extracellular vesicles derived from cardiac cells in
an in vitro model of preconditioning. Journal of Extracellular Vesicles 6 (1): 1390391, DOI:
10.1080/20013078.2017.1390391
Brylka, L.J.; Köppert, S.; Babler, A.; Kratz, B.; Denecke, B.; Yogan, T.A.; Etich, J.; Costa,
epiphysiolysis causes distal femur dysplasia and foreshortened hindlimbs in fetuin-A
deficient mice. PlzG Ont: 0167030. [IF 2016: 2.806]
Rose, M.; Meurer, S.K.; Kloten, V.; Weiskirchen, R.; Denecke, B.; Antonopoulos, W.;
Deckert, M.; Knüchel, R.; Dahl, E. (2017) ITHS induces a shift in TGF-β1 superfam-
ily signaling involving Endoglin and reduces risk for breast cancer metastasis and tumor death.
Mol Carcinog.:167-181. [IF 2016: 4.185]
Gan, L.; Denecke, B. (2017) Co-regulation of microRNAs and transcription factors in
cardiomyocyte specific differentiation of murine embryonic stem cells: An aspect from
[IF 2016: 5.016]
Mellows, B.; Mitchell, R.; Antonioli, M.; Kretz, O.; Chambers, D.; Zeuner, M.T.; Denecke,
B.; Musante, L.; Ramachandran, D.L.; Debaq-Chianaux, F.; Hoffhoffer, H.; Joch, B.; Ray,
and Molecular Characterization of a Clinically Compliant Amniotic Fluid Stem Cell-Derived
Extracellular Vesicle Fraction Capable of Accelerating Muscle Regeneration Through
Enhancement of Angiogenesis. Stem Cells Dev.: 1316-1333 [IF 2016: 3.69]
Garczyk, S.; Klotz, N.; Szczepanski, S.; Denecke, B.; Antonopoulos, W.; von Stifffried,
cancer are associated with NMU-R expression involving crosstalk with members of the
Core Facilities | Immunohistochemistry Facility

---

**Head of the facility**
Ensslen, S.

**Advisory Board**
Ostendorf, T.
(Clinic for Renal and Hypertensive Disorders, Rheumatological and Immunological Diseases (Medical Clinic II))

**Skills/Services/Training courses/Consulting service**
At the immunohistological facility of the IZKF Aachen researchers can order all technical service necessary for histological and immunohistological experiments.

The following service can be offered:
- pre-experimental consulting
- support to excision and preparation of samples and tissues (choice of proper fixatives)
- dehydration and embedding of fixed tissues
- preparation of tissue slices (paraffin and cryo)
- histological staining (H&E, EvG, Giemsa, PAS etc.), direct and indirect immunohistological staining
- testing of antibodies (according to prior agreement)
- microscopy and documentation (according to prior agreement)

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**Funding period:** 01.07.2017 - 30.06.2020

**Staff:**
- TV-L14: Ensslen, S.
- TV-L9: Tappe, M.
- TV-L8: Wüffel, J.

**Materials/Travel expenses 2017:** € 19,092

**Investments/Equipment 2017:** € 3,200

**Revenues 2017:** € 28,501

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Status and Development
In 2017 the IHC facility improved the quality control. After we invested in equipment to automate different procedures we now invested in further training of the laboratory assistant in quality management. That enables us to provide high quality service in all fields concerning histology/immunohistology.

Users within the faculty in 2017
117 researchers from 27 different clinics/institutes of the medical faculty at the RWTH Aachen and 5 researchers from 2 extern institutes used the immunohistological facility. A total of 19711 tissue samples were dehydrated and embedded. 14058 slices were produced and 2752 slides were stained. 200 immunohistological staining procedures were performed.

Status and Development
Confocal microscopy generates sharp images from fluorescently labelled samples. Out-of-focus light that blurs the image in conventional microscopy cannot pass the confocal pinhole and is therefore eliminated. From serial optical sections a 3-dimensional representation of a sample can be generated. Confocal microscopy is performed on tissue sections, fixed cultured cells or even with living cells containing an appropriate fluorescence label. Laser light is used for efficient excitation of fluorescently labelled samples. The various lasers of a confocal microscope can also be used as a precision tool to modulate fluorescence in living cells with subcellular resolution. Based on this property, advanced fluorescence techniques have emerged that can give access to the intracellular dynamics and interactions of fluorescently labelled biomolecules.

Confocal Microscopy can be used for the generation of confocal images of fixed samples and the investigation of living cells with advanced fluorescence techniques. Offered services are the following:

Confocal images:
• Pre-experimental consulting and hands-on training
• 2-dimensional confocal images
  – Multi-channel images with up to 4 fluorophores
  – Exact overlay of with differential interference contrast (DIC) images
  – Fluorescence intensity profiles
  – Colocalization analysis
• 3-dimensional confocal images
  – Preparation of image stacks (z-stacks)
  – Reconstruction of 3-dimensional objects
• Advanced microscopy:
  – Pre-experimental consulting and hands-on training
  – FRAP (fluorescence recovery after photobleaching) and FLIP (fluorescence loss in photobleaching) to determine the mobility of fluorescently labeled molecules in living cells
  – FRET (Förster resonance energy transfer) to detect interaction of fluorescently labeled molecules
  – BiFC (bimolecular fluorescence complementation) to detect interaction of specifically labeled molecules
  – Use of photoconvertible and photointeractivatable fluorescent proteins to determine protein dynamics in living cells
  – Live cell imaging with temperature- and CO2-control

Head of the facility
Müller-Newen, G. (Institute of Biochemistry and Molecular Biology)

Advisory Board
Lüscher, B. (Institute of Biochemistry and Molecular Biology)

Skills/Services/Training courses/Consulting service

Confocal Microscopy Facility

Funding period: 01.07.2017 - 30.06.2020

Staff:
TV-L13 (65%): Ernst, S.

Materials/Travel expenses 2017: € 17,067
Investments/Equipment 2017: € 1,108
Revenues 2017 € 0
Status and Development
With the beginning of 2018 the Confocal Microscopy Unit is since six and a half years part of the IZKF Core Facilities. In 2017 confocal microscopy has been used by 19 institutes and clinics of the Medical Faculty. In most cases, more than one individual of the respective department was involved as a user (see table below). Thus, the number of users exceeds 40. At least 3 papers have been published in 2017 with support by the IZKF Core Facility mentioned in the acknowledgement section (refs. 1-3). In context of the Art. 91b GG application by Dr. Rafael Kramann and colleagues a new confocal & super-resolution microscope will be installed within the facility in early 2018.

Users within the faculty
42 user from 17 different clinics/institutes of the medical faculty at the RWTH Aachen used the Confocal Microscopy Facility.

Collaborations
The Confocal Microscopy Facility has a collaboration with Grünenthal GmbH.

Publications


Applied and actual third-party funding
Kramann, R. and colleagues including Müller-Newen, G. Laser Scanning Confocal Microscope with Super-Resolution DFG/NRW 07/2017-06/2018 € 475,000
**Head of the facility**
Mingoia, G.

**Advisory Board**
- Binkofski, F. (Neurology Clinic)
- Mathiak, K. (Clinic for Psychiatry, Psychotherapy and Psychosomatic Disorders)
- Habel, U. (Clinic for Psychiatry, Psychotherapy and Psychosomatic Disorders)
- Schneider, F. (Clinic for Psychiatry, Psychotherapy and Psychosomatic Disorders)
- Wiesmann, M. (Clinic for Diagnostic and Interventional Neuroradiology)

**Funding period:** 01.07.2017 – 30.06.2020

**Staff:**
- TV-L 14: Mingoia, G., Zvyagintsev, M.
- TV-L 13: Schüppen, A.
- TV-L 13 (100%): Shah, D.
- TV-L 11: Buchner, D.
- TV-L 9 (50%): Eder, G.
- SHK: Richter, F., Devi, R., Funkner, A.

**Materials/Travel expenses 2017:** € 14,661

**Investments/Equipment 2017:** € 31,442

**Revenues 2017:** € 53,725

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**Skills/Services/Training courses/Consulting service**

**Our services**
- Neuroimaging experiment preparation
- Paradigm optimisation, preparation and programming
- Project presentation: Neuroimaging Colloquium
- Engineering of equipment for neuroscientific experiments
- Setup and optimisation of MRI protocols
- MRI and PET measurements
- MTA support for conducting MRI-based experiments
- Real-time fMRI and Neurofeedback support
- Hyperscanning fMRI by using three Prisma Siemens scanners
- Support, maintenance and optimisation for all equipment installed at MR scanners in-house
- Physiological signals (ECG, EDA, etc.) & fMRI: simultaneous acquisition
- Neuropsychological/behavioral data acquisition
- Neuropsychological workstation in a light/sound shielded space
- Data storage
- MRI data conversion, pseudonymization and assessment
- Database and long term storage of raw data
- Data analysis
- Quality assurance of the acquired data
- Users' computer account with redundant back-up
- Data analysis and statistical computations with numerous software packages on the BIF's servers & computers
- Preparation, presentation and publication of results
• Theoretical courses of the main analysis methods. The BIF provide following training courses: Presentation® course (D. Buchner), MATLAB® course (A. Schüppen), SPM courses (A. Schüppen), Brain Voyager® course (M. Zvyagintsev), Linux course (D. Buchner). Our members are involved in several course provided for students of the RWTH.

• Neuroimaging colloquium. A fortnightly meeting where new experiments are presented.

• Principal investigator meeting. A monthly appointment where the development strategies and needs of particular research groups are discussed.

• The detailed list of our activities is presented at our WEB site; (https://www.izkf.rwth-aachen.de/ webmaster G. Mingoia). The site also contains information about the equipment, software, services, lectures, conferences and responsibilities of the BIF members.

Status and Development
Beyond keeping on course noteworthy number of services, the BIF initiated in 2017 a series of technical and methodological developments consistent with the triennial funding plan accepted by IZKF board in 2017. The improvements concerned both new investments, made possible by revenues budget, and the offer of additional services also thank to the hiring of new personal.

Comprehensive MRI quality assurance
The development of automatized and scripted signal processing and comprehensive quality assurance is essential for collaborative and large-scale projects. In 2017 we have realized in collaboration with the “Wissenschaftliche Werkstatt” of medical faculty an internal coil support for imaging phantom compatible with our 31P (Phosphor) and 23Na (Natrium) head coils that will permit to perform more precise calibration for spectroscopy MRI measurements; quality assessment results are reported weekly on our web-site. The BIF collaborates with research groups in order to clean data from physiological noise using two Biopac® MP150 MRI compatible systems with AcqKnowledge® Software purchased at the end of 2016 (~40.000 €); the BIF will collaborate in the development of this device providing its experience and feedback from users to NNL.

MRI goggle system with integrated eye tracker
As announced in the 2017-2020 funding plan the BIF has decided to invest again in goggle system to provide visual stimuli to subjects/patients during fMRI measurements. For this purpose the BIF started a collaboration with Neuro Nordic Lab (NNL) which will provide their most advance goggle system; all the cost will be supported by NNL (~30.000 €); the BIF will collaborate in the development of this device providing its experience and feedback from users to NNL.

Walking machine for Siemens Prisma MRI scanner
New frontiers of fMRI research expand in many challenging direction like observing brain activity during real walking action. In order to permit subjects to walk inside MRI bore, we have realized in collaboration with the “Wissenschaftliche Werkstatt” of medical faculty a walking machine 3T MRI compatible fully adjustable that can be installed on the bed of Prisma scanners.

Gustatometer
According to the wish of principal investigators we have decided to acquire a fully programmable 7+1 channel MRI compatible Gustatometer from “Emerging Tech Trans, LLC” with subject feedback and sub-second data resolution for fMRI and electrophysiological studies of the gustatory system (~30.000 €).

MRI compatible headphone and microphone with active noise cancellation
An OptoActive two-way ear selectable noise cancellation from Optoacoustic (37.766,14€) was bought at the beginning of 2017. During 2017 the BIF set-up this system in order to permit our collaborator to take advantage of all its potentiality.

Neuroimaging colloquium
Finally, the BIF consolidates its central position in the scenario of Neuroimaging research. It represents the home for neuroimaging research in the Faculty of Medicine of the RWTH Aachen, and the meeting point is well represented by our neuroimaging colloquium, which consisted of 22 appointments only in 2017 (all reported on our web-site).
Users within the faculty

85 users from 6 clinics/institutes of the medical faculty used the Brain Imaging Facility. The main users are the Clinic for Psychiatry, Psychotherapy and Psychosomatic Disorders.

Collaborations

The Brain Imaging Facility has project collaborations with the Clinic of Psychiatry and Psychotherapy of the University of Jena, the Brain Innovation, Maastricht (Netherlands), the Royal University of Leuven (Belgium), the University of Minnesota, USA, the University of Freiburg (Germany), Siemens (Germany), Research Center Jülich, Resonance Technology, USA, the Emerging TechTrans, USA, ETH/UZH - NeuroNordicLab AS from Sweden and the Optoacoustics, Israel.

Publications


Repple J, Pawlczek CM, Voss B, Siegel S, Schneider F, Kohn N, Habel U (2017) From provocation to aggression: the neural network. BMC Neuroscience. [IF 0.94]


Kellermann T, Scholle R, Schneider F, Habel U (2017) Decreasing predictability of visual motion enhances feed-forward processing in visual cortex when stimuli are behaviorally relevant. Brain structure&function. [IF 3.4]


Bzdok D, Kamer TM, Habel U, Schneider F (2017) Big data approaches in psychiatry: examples in depression research. Nervenarzt. [IF 0.45]


Panagiotidis D, Clemens B, Habel U, Schneider F, Schneider I, Wagels L, Voltvov M (2017) Exogenous testosterone in a non-social provocation paradigm potentiates anger but not behavioral aggression. European Neuropsychopharmacology. [IF 0.23]


Two-Photon Imaging Facility

Head of the facility
Vogt, M.

Advisory Board
Tolba, R. (Institute of Laboratory Animal Science)
Martin, C. (Institute of Pharmacology and Toxicology)
van Zandvoort, M. (Institute for Molecular Cardiovascular Research)

Imaging techniques such as magnetic resonance imaging, computer tomography, positron emission spectroscopy, or ultrasound, enable non-invasive visualization of various functional and structural aspects of samples. However, the spatial resolution of these imaging modalities does not allow visualization of subcellular structures. Moreover, they sometimes lack the specificity that is required for visualization of delicate molecular properties of samples. These requirements are met by optical fluorescence microscopy which combines subcellular resolution (<1 µm) with molecular selectivity and sensitivity. However, classical fluorescence microscopic techniques visualization of structures deep in intact viable samples cannot be performed, even when using optical techniques such as laser scanning confocal microscopy. Two-photon laser scanning microscopy (TPLSM) however, meets the requirements for studying intact viable samples at subcellular resolution.

TPLSM is based on the principle of two-photon excitation where simultaneous absorption of two near-infrared photons (total energy is equivalent to that of a single photon at half the wavelength as used in classical fluorescence microscopy) leads to the excited state of fluorescent molecules in the sample. Since the likelihood of simultaneous absorption (within ~ 0.5 fs) of two photons is very low, excitation only occurs in the focus of a pulsed near-infrared laser. Out of focus absorption and excitation are absent leading to imaging of an optical slice. Furthermore, near-infrared light penetrates deeper into scattering tissue allowing the excitation deep in the sample. Scanning a stack of images in a definite depth range enables a 3D reconstruction and the spatial visualization of fluorescent structures in the imaged volume. As a result, TPLSM possesses enhanced depth penetration, good optical sectioning, and good resolution in three dimensions. Moreover, photo-bleaching, photo-damage and phototoxicity are reduced outside the area of interest. The combination of all these features makes TPLSM advantageous over other microscopic techniques for visualization of structures located deeper in viable scattering or vulnerable tissues in three dimensions.

The two-photon core facility is equipped with two two-photon laser scanning microscope systems. The LaVision BioTec TrinSMcpe I microscope was adapted to higher speed scanning by a bidirectional scan mode suited for in-vivo imaging. The motorized sample table of the LaVision TrinSMcpe enables the sequential imaging of different areas in the same sample for an effective study of time- and cost-consuming experiments. Furthermore, the fluorescence filter set offers a high flexibility in the imaging of fluorophores. The second microscope system (Olympus Fluoview FV1000 MPE) is the best choice when maximal penetration depth is required because of its highly efficient optics and its powerful laser. Moreover, its flexible layout (3 internal and 4 external detectors) and the automated handling allow the simultaneous detection of a wide range of fluorescent molecules and the independent usage of trained researchers. The two-photon microscope systems can be used for in-vitro, ex-vivo, in-situ, and in-vivo experiments and are mainly prepared for imaging in rodents or isolated “whole mount” samples, but also for in-vitro samples or tissue slices. Furthermore, we offer the option for animal triggered in-vivo imaging, in which ECG and respiration signals are used to limit the impact of motion disturbances.
The In-Vivo Optical Imaging systems (Perkin Elmer IVIS Lumina XR/ Berthold NightOWL) enable the recording of bioluminescence and fluorescence signals from cell cultures, ex-vivo samples and non-invasively in living small animals for extended time periods in the same animal. The overlay with a photographic or radiographic image brings the bioluminescence and/or fluorescence signal into an anatomical context.

Status and Development
In 2017 the microscope systems have been successfully used by a large number of projects. The use of the innovative method of optical tissue clearing (established by Dr. V. Puelles-Rodriguez, Med. Clinic II) was further extended and transferred to other users of the facility. To utilize the full potential of large tissue cleared samples, a new motorized sample stage was purchased for the Olympus FV1000MPE system. The motorized stage enables the spatial imaging of large sample areas in high resolution using mosaic scanning and image stitching. The input power of the excitation laser is a crucial factor for evaluating the light burden in the sample and the correct laser beam adjustment. To measure this parameter a laser powermeter was added to the Core Facility equipment.

Current projects and the establishment of innovative methods (e.g. tissue clearing) also show the limitation of the systems in speed, sensitivity and penetration depth. Despite the efforts to keep the TPLSM systems up-to-date, the possibilities for further development of the devices, due to their age (2008), are limited. In particular, progress in the area of long-wave dyes and fluorescent proteins (transgenic animals) cannot be covered by the current systems.

To offer a state-of-the-art two-photon microscope system to all researchers of the medical faculty the acquisition of a new system in the future should be considered. A future investment in a new microscope with the option for infrared imaging (>1000 nm excitation) would allow the establishment a dual imaging platform by combining in-vivo optical imaging and TPLSM. An experimental set-up from whole-body imaging to subcellular imaging deep in the tissue in the same animal would be a unique feature at RWTH Aachen University. The methodological scope of the Core Facility could be enriched by an excellent addition.

Users within the faculty
43 users from 8 clinics/institutes of the medical faculty used the Two-Photon-Imaging Facility. The main users are Department of Medicine III, Clinic for Dental Prosthetics and Biomaterials, Implantology Centre and the Biomedical technologies - Institute of Experimental Molecular Imaging.

The generation of big imaging data is followed by the need of hard- and software to visualize and process the data. Therefore the Core Facility offers three high-performance workstations and image processing software (e.g. ImageJ/FIJI, ImagePro, Imaris, AutoQuantX3) for data processing and analysis. The image processing software Imaris (Bitplane) was upgraded in 2017 on two workstations. In addition to local storage, the workstations of the Core Facility are connected to a data server (IZKF-Cloud) for temporal storage. The users of the Core Facility have decentralized access to their microscopic data for data processing and transfer.

The Team of the Two-Photon Imaging Facility.
Core Facilities | Two-Photon Imaging Facility

Collaborations
The Two-Photon-Imaging Facility has project collaborations with the Advanced Microscopy Unit (CARIM), University Maastricht (the Netherlands) and the Institute for Cardiovascular Prevention of the LMU Munich.

Publications


Core Facilities | Transgenic Service

The core facility “Transgenic Service” (TGS) supports all members of the medical faculty at the university hospital of the RWTH Aachen as well as the RWTH Aachen University with the generation of genetically modified (transgenic or knockout) mouse strains. Thereby, cloned DNA sequences are transferred into the genome of the animals. Depending on the scientific aim the inserted DNA sequence is translated into a biologically active protein or a certain gene of the recipient animal is modified or knocked out. For the generation of genetically modified mice (e.g. as model system for human diseases) two methods, pronuclear injection and homologous recombination are predominantly used.

A main task of the Transgenic Service is the organization, the cryopreservation, revitalization and rederivation of existing mouse lines for hygienic or strain-conservation reasons. Another focus of the core facility is the support of the different research groups in the breeding management and consultancy in projects in regard to the 3R principles of Russel & Burch 1959 (replacement, refinement and reduction of animal experiments). The Services of the Transgenic Service contain following items.

1. Embryo transfer and rederivation of infected or imported mouse lines
2. Cryopreservation of mouse embryos and mouse sperms
3. Quality control of frozen mouse embryos and mouse sperms
4. Generation of knockout animals
5. Generation of transgenic animals
6. In-vitro fertilization (IVF)
7. Intra cytoplasmatic sperm injection (ICSI)
8. Cell culture of embryonic stem cells
9. Import and export management
10. Organization of our animal software database “Tierbase”
11. Organization of animal capacity in our animal facilities
12. Breeding management and consulting service for experimental setup according to the 3R principles of Russel & Burch 1959 (Replacement, Refinement and Reduction of animal experiments)
13. Administration of surgery training in FEELASA B courses
14. Share of the responsibility in the education of animal care taker in teaching and research

Funding period: 01.07.2017 - 30.06.2020

Staff:
TV-L9 (50%): Pfeffer, T., Lingg, F., Pietsch, K.
TV-L9 (100%): Jung, J.

Materials/Travel expenses 2017: € 14,010

Animal-Housing Costs 2017: € 4,771

Investments/Equipment 2017: € 61,270

Revenues 2017: € 58,241
Pronucleus injection:
Transgenic DNA constructs are injected in the pronucleus of fertilized eggs. These DNA constructs can contain species-specific genes or genes from another species (e.g., human) under control of species-specific or foreign promoters. The transgenic embryos are transferred into pseudopregnant recipient mice. A part of the newborn mice has integrated this transgene into the host genome. These founders are then used for subsequent transgenic breeding to establish the transgenic mouse line.

Homologous recombination (HR):
Murine embryonic stem cells (ES cells) containing the modified (knockout/knockin) allele are microinjected into mouse embryos (morulae or blastocysts). Due to their germ line competence these cells can pass the modified knockout allele to the next generation, starting with a chimeric founder. Knockout/knockin mice are very suitable for elucidating gene functions and may also be used as reporter strains.

Cryopreservation of mouse embryos and mouse sperms:
Fertilize embryos are isolated, handled in special media and are frozen by the rapid freezing method in a propylene glycol medium. The embryos are cooled down by a decrease in temperature of 0.3 - 0.8°C/min stepwise to -30°C and transferred then immediately into liquid nitrogen (-196°C) for long time storage. This method reduces the crystallization inside the embryos and thereby increasing the survival rate after storage and thawing. Another method to preserve mouse lines permanently is the freezing of mouse sperms. The sperms are isolated from the epididymis of a male donor and frozen in a special cryoprotective medium. At first, the sperms are frozen slowly in the gas phase of liquid nitrogen and then transferred into liquid nitrogen.

Two liquid nitrogen tanks (capacity 300,000 embryos/tank) are available for long time storage. The cryopreservation of mouse strains strongly promotes the 3R principle (Replacement, Reduction, Refinement) for animal welfare (here: reduction) by avoiding the production of mice that are not currently used and thereby also help decreasing the animal housing costs for the users. Furthermore, shipment of frozen embryos or sperms instead of living animals simplifies the distribution of interesting mouse strains and allowing the direct sanitization by embryo transfer upon arrival.

In Vitro Fertilization (IVF):
- The IVF has become an important method in the service routine of
- Rederivation
- Cryopreservation
- Revitalization

Unfertilized eggs from hormone stimulated donor females are incubated in a specific medium with frozen or fresh sperms in an incubator. The obtained fertilized oocytes (zygotes) can be implanted in a pseudo pregnant female (for rederivation or revitalization) or the zygotes can be used for cryopreservation.

Because of the low number of donor females, IVF is the preferred method for the cryopreservation of mouse embryos. Thereby IVF decreases the service cost for the researcher, but also follows the 3Rs (reduce, refine, replace) to reduce animal numbers.

IVF has also become an important part in the rederivation of mouse lines for hygienic or strain-conservation reasons in our SPF-barrier (specific pathogen free).

Instead of importing living animals with a potential risk of infections (according to FELASA Guidelines), it is possible to buy frozen sperms which are revitalized by IVF. Another possibility to reduce the risk of infections is the import of epididymides of donor males. The donor females are bought by commercial companies and held in our quarantine facility until the experiments. Alternative to be able buy live animals in consideration of the health status and keep the mice however the donor female in the quarantine facility.
Embryos are retrieved from superovulated, mated females, and are immediately transplanted in clean, health-certificated foster mice.

After weaning and genotyping of the offspring from IVF or injection routines, the foster mouse is subjected to health monitoring according to the guidelines of the Federation of European Laboratory Animal Science Associations (FELASA).

Status and Development
The Transgenic Service continues to be an important partner for researchers of the life science faculties (medicine, biology) of the RWTH as well as for external collaborators. In 2017 the Transgenic Service could increase the productivity, especially for the service cryopreservation of mouse sperms.

The import of genetically modified mouse lines increased substantial. The hygiene status of these imported animals is often a risk for the health status of the breeding in the animal facility. Therefore rederivations of the animals must be accomplished. For this purpose the mice are hormone-stimulated (superovulation). Since these animals are potentially infectious, this work may not be accomplished by people, who work in the SPF (specific pathogen free) facility. Because of this hygienic reason it is necessary to employ three student helpers (SHK) for this routine work. These positions are financed by third party funds of Univ.-Prof. Tolba.

Moreover, to ensure the quality of our services and the claims of internal and external users, the core facility “Transgenic Service” as a part of the institute of laboratory animal science, has been certificated according to DIN ISO 9001:2015 in 2012.

In cooperation with the core facility „Two photon Imaging“, the skills of the TGS is an ideal addition to extend the scope of biomedical research in the medical faculty. The existence of a state of the art core facility „Transgenic Service“ for genetic manipulation of animal models is from there a crucial element to guarantee the future of biomolecular research and laboratory animal science at the RWTH Aachen university.

The experiences of the last two funding periods show that the successful work of the core facility has a tremendous impact for the medical faculty, e.g. for professorship appointments and acquiring third-party funds (e.g. SFB TRR 57). It is thereby a central component for the biomedical research at the science location Aachen and an enormous competitive advantage.

Existing users but also new research groups took advantage of the current services in the last year. At present a further development of the offered services in the core facility is not planned due to the mouse breeding capacity as well as the limited personnel positions and laboratory space.

Since 02.01.2018, Elke Brehm supports the Transgenic Service team with 50% of her working time.

Resulting costs including equipment maintenance, investments etc. could be covered by revenues from the projects.

Users within the faculty
32 users of 19 institutes/clinics of the medical faculty used the Transgenic Service. The main user is the Department of Internal Medicine III.

The Transgenic Service had two external users from the University of Maastricht and the University of Newcastle.
Core Facilities | Proteomics Facility

Head of the facility
Preisinger, C.

Advisory Board
Lüscher, B. (Department of Biochemistry and Molecular Biology)
Schulz, J. B. (Department of Neurology)
Saez-Rodriguez, J (COMBINE)

Skills/Services/Training courses/Consulting service

Sample preparation:
- The facility provides advice on sample preparation (tissues, organs) in order to ensure appropriate protein extraction
- The actual sample preparation (including lysis and downstream processing is usually carried out in the proteomics facility (the chosen procedure depends on the “state” of the submitted sample (s)).
- Gel electrophoresis of protein samples
- Proteolytic digestion of protein samples in-solution, in-gel or on-bead (proteases: Trypsin, LysC, GluC, ...)
- Sample purification and desalting (C18; small (zip-tips) to large scale (cartridges)
- Sample labelling: If protein quantitation is required within the particular project, peptides can be isotopically labelled using the dimethyl stable isotope labelling method. Cells that have been grown in SILAC medium can be used as an alternative.

Sample enrichment - peptide chromatography:
- The facility provides peptide separation using high pH reversed phase chromatography or similar techniques such as strong anion/cation exchange (SAX/SCX) in both small scale (pipette tip format) as well as large scale (HPLC).
- The facility also provides phosphopeptide enrichment using TiO2/Ti-IMAC based chromatographic technology (for serine and threonine phosphorylation) or anti-phosphotyrosine antibody based immunoprecipitations of tyrosine-phosphorylated peptides and subsequent quantification of phosphorylation changes.

Mass spectrometry:
- Analysis of purified samples by nanoLC-MS/MS. Samples are analysed on the Orbitrap Elite system as well as the new Q Exactive Plus system.

Data analysis:
- The analysis of the raw data is performed in house using designated software packages (MaxQuant (with the built in Andromeda search engine), PeptideShaker and Perseus). This includes protein (and PTM) identification and their relative quantification. The resulting data is explained to and discussed with the researchers. Furthermore, the facility provides advice on data interpretation and possible follow-up experiments.
- Storage of raw data in house (IZKF cloud) on separate hard drives for access at later time points.

Special services:
- The facility can also provide protein electrophoresis and western blot analysis for groups that are not equipped with the required instrumentation.
- We also provide technical assistance and expertise for “unusual” biological questions in the field of protein biochemistry, including non-common protein chromatography and in vitro assays.

Status and Development
The Proteomics Facility was launched in January 2012 in order to provide the medical faculty of the RWTH Aachen with access to proteomic technologies in order to facilitate the analysis and investigation of protein function on a molecular level in health and disease. This has been well received throughout the entire faculty and attracted a number of interested groups among both pre-clinical institutes and clinical departments. The medical faculty of the RWTH Aachen enabled the acquisition of a refurbished high resolution/high mass accuracy MS (Orbitrap Elite Thermo Scientific) in 2013 in order to facilitate and improve the proteomic research of the faculty. This investment was necessary due to the outdated equipment present in the proteomics core facility at that time. The implementation of the new MS was met with both strong enthusiasm as well as high expectations amongst many research groups. This Orbitrap instrument enabled the facility to drastically increase...
the availability of experimental approaches that could be utilized to analyse protein/proteome samples in the past years. In particular, it was successfully used for the analysis and characterization of large-scale protein interactomes and the quantitative analysis of protein phosphorylation changes in collaboration with several groups within the medical faculty. This work has resulted in several high impact publications by different research groups of the faculty where the proteomics core facility provided substantial input over the past years, with several more manuscripts currently in revision/preparation. Furthermore, several grant applications (such as DFG grants or larger consortia), with the proteomics facility providing key technology to the individual projects, have been awarded or are under review/in preparation. Additionally, the proteomics facility will play a key role in the newly funded IZKF project "Mesenchymal interactions and fibrogenic signalling in cancer development: Molecular mechanisms in solid and hematologic neoplasms" (Joint project funding of the research area "Oncology"; speaker Prof. Dr. Ralf Weiskirchen). Within this multidisciplinary consortium the proteomic facility will contribute to many individual projects. As a logical consequence of these recent developments, the demand for high resolution/high mass accuracy mass spectrometry in the faculty has again severely increased. Based on intense combined efforts of the proteomic facility, many of its dedicated users, the IZKF and the medical faculty it was possible to acquire a new mass spectrometer in December 2017. The new instrument (Q Exactive Plus (Orbitrap instrument; Thermo Scientific)) will be operational in Q2 2018, enabling a significant increase in proteomic analysis possibilities within the faculty. This will include the analysis of tissues/organs from animal models of various diseases (including transgenic mouse and fly models) as well as human samples (such as freshly derived biopsies) and protein samples obtained from cell culture approaches. Furthermore, the new instrument will enable the usage of both isobaric labelling as well as label-free quantification, therefore expanding the availability of proteomic quantification approaches. The proteomic facility will thus aid in the qualitative and quantitative analysis of both complete proteomes (such as organs, tissues or similar) as well as the investigation of post-translational modification (PTMs, mainly modification of amino acids by phosphorylation) throughout the following years.

Collaborations
The Proteomics Facility has project collaborations with the Institut of Pathology - Functional proteomics research group of the Medical University of Graz (Austria), the MRC Institute of Genetics & Molecular Medicine of the Cancer Research UK Edinburgh Centre and the FH Aachen.

External users
- Dr. Ferdinand Kappes, Department of Biological Sciences/Xi’an Jiaotong-Liverpool University
- Dr. Olaf Neumann, University Clinic Heidelberg, General Pathology
- Dr. Federico Pinna, University Clinic Heidelberg, General Pathology

Publications

Applied and actual third-party funding
Preisinger, C. Phosphoproteomic analysis of the tyrosine kinase inhibitor Ponatinib Ariad Pharmaceuticals 07/2015-06/2017 € 10,000
Preisinger, C. Mass spectrometry analysis of the phosphoproteome of mast cell leukemia cells: curative effects of tyrosine kinase and MEK inhibitors Leonards Stiftung 05/2016-04/2017 € 5,000

Collaborations
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External users
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- Dr. Federico Pinna, University Clinic Heidelberg, General Pathology

Publications

Applied and actual third-party funding
Preisinger, C. Phosphoproteomic analysis of the tyrosine kinase inhibitor Ponatinib Ariad Pharmaceuticals 07/2015-06/2017 € 10,000
Preisinger, C. Mass spectrometry analysis of the phosphoproteome of mast cell leukemia cells: curative effects of tyrosine kinase and MEK inhibitors Leonards Stiftung 05/2016-04/2017 € 5,000

Users within the faculty
38 users from 14 clinics/institutes of the medical faculty used the Proteomics Facility. The main users are Department of Internal Medicine II and the Institute of Biochemistry and Molecular Biology.
Flow Cytometry Facility

Head of the Facility
N. N.

Advisory Board
Pabst, O. (Institute of Molecular Medicine)

Skills/Services/Training Courses/Consulting Services

The FCF supports Flow Cytometry (fluorescence-activated cell sorting - FACS) based cell purification and analysis. Flow Cytometry is the gold-standard technology for purification and analysis of cells with complex phenotypes. However, the complexity of FACS technologies creates hurdles for the individual scientist to make full use of these techniques. The FCF enables use of flow cytometry across disciplines. The FCF is open to all members of the medical faculty of RWTH Aachen; depending on free capacities scientists from other research institutions are also welcome to use our services.

Support comprises all stages of FACS based experiments: from designing the most promising experimental strategy, benefit from state of the art cell protocols and access to well-tested reagents, to the point of fully serviced cell purification and help with FACS based analysis, interpretation of data and protocol development.

- individual advise in designing flow cytometry based experiments
- advise in sample preparation by providing state-of-the-art protocols for cell preparation from mouse and human tissues and cell staining
- access to antibodies to allow for proper compensation and control of staining specificity
- hands-on training on FACS analysis using BD FACS Canto. The FACS Canto is open to all users and allows complex multi-colour analyses of cell populations.
- cell sorting under safety level S1 conditions as a service according to user demands
- advice in interpretation of experimental data
- in near future CFFC will offer courses and seminars informing about opportunities, basic principles and recent advances in flow cytometry.

Funding period: 01.07.2017 - 30.06.2020

Staff:
TV-L 9: Steinke, N.

Materials/Travel expenses 2017: € 11,318
Investments/Equipment 2017: € 21,152
Revenues 2017: € 0

Status and Development

Status quo: Since 2014, members of the Institute of Molecular Medicine have supported on a voluntary basis artists at RWTH Aachen University in performing FACS based experiments. In particular Hildegard Ostendorp has performed cell sorting experiments and Dr Vuk Cerovic and Prof Oliver Pabst have consulted colleagues in the use of flow cytometry. These efforts have been channelled and concentrated in the new flow cytometry facility supported by the IZKF since 07/2017. Thus, FCF is the newest core facility supported by IZKF.

The FCF is temporarily headed by Prof. Oliver Pabst. To run the facility a new technician funded by FCF/IZKF, Nathalie Steinke, has been recruited in December 2017 (no personal was paid by CFC/IZKF before December 2017). With the help of the Institute of Molecular Medicine Mrs. Steinke has been trained to operate the cell sorter. Nathalie Steinke coordinates the schedules, operates the cell sorter and maintains the facilities' technical equipment. We expect that by summer 2018 Mrs. Steinke will have learnt a broad range of flow cytometry techniques and will be able to handle complex experiments. In February 2018 the position of FCF head has been announced. We aim to recruit a highly motivated scientist to head the facility by summer 2018. At this step Prof. Pabst will stop to coordinate FCF activities.

To physically set up the facility the Institute of Molecular Medicine provides lab space. Dedicated FCF lab space is now on level 5, floor 41, room 7. This room is open from Monday to Friday, 8:00 a.m. until 4 p.m. Outside the main working hours, users can access the room by PIN code. A PIN secured safe to lock the respective key for the FCF room has been installed in the floor directly outside the FCF room.

The FCF currently operates two FACS instruments. A Becton Dickinson (BD) FACS Canto Analyzer equipped with three lasers and detection of up to eight fluorescence parameters and a BD FACS Aria cell sorter equipped with three lasers allowing the detection and sorting of particles defined by up to nine fluorescent parameters.
The Analyser (BD Canto) had previously been installed at the central IZKF lab space and now has been moved into the FCF lab. The BD FACS sorter had been upgraded by funds provided by the Institute of Molecular Medicine and has been moved to the FCF lab space in December 2017.

Funds budgeted for the FCF technician from 07/2017 until 11/2017 (the vacant position of a technician from July 2017 until December 2017, before Mrs Steinke had been employed) were used to purchase basic equipment. In particular the facility had been equipped with a laminar flow/safety cabinet, basic lab equipment such as pipettes and a centrifuge and three Computers. This step has moved to FCF into a largely independent position and the FCF can handle many activities without external equipment. The facility is still dependent on resources of the Institute of Molecular Medicine with respect to office space, storing capacities, administrative support, cooling devices and more dedicated lab equipment e.g. for cell isolation or sophisticated staining protocols.

The FCF has set up documents to explain facility rules and services (flyer and homepage). Prof Pabst has started to assemble documents for a DFG grant to purchase a new cell sorter (by now colleagues have submitted their research proposals and CVs, quotes have been collected from the main provider of FACS instruments, the grant shall be submitted once the homepage has been finalized and is online).

Now that FCF has its own personal, Nathalie Steinke, we have documented use of the FCF and provided cell sorting services. Starting January 2018 the FCF will charge users as set forward in the user regulations.

In the first two months (December 2017/January 2018 - since we record use of FCF in detail) the FACS Canto analyser was used by 22 scientists coming from eight institutes/clinics and cell purification service was used by ten scientists from six institutes/clinics).

**Future development:**

Since no funds and office/lab space are officially assigned to the FCF, we expect that the Institute of Molecular Medicine will need to continue subsidising the FCF (lab and office space, administrative support, lab equipment) for the near future. Recruitment of the acting facility head will be key for the future development of the FCF.

With the aim to motivate experienced and independent scientists to apply for this position, the Institute of Molecular Medicine is prepared to offer the future head of the facility full access to its resources. Thereby, we hope to recruit a highly motivated person who will be able to recruit external funds, follow independent research questions and establish novel methods/tools/FACS technologies. Funding of the position as head of the FCF will be available starting July 2018 (as it had been applied for in the initial proposal).

Support by a full scientist will greatly expand the services and opportunities in the FCF. Most notably we will be able to 1) provide courses and more intense personal training in flow cytometry 2) develop methods according to user demands 3) set up regulations and instrumentation to handle primary human cells and S2 material 4) expanded the FCF portfolio.

**Support by a full scientist will greatly expand the services and opportunities in the FCF.**

1) Courses and more intense personal training in flow cytometry. We realized that many users do not make full use of this technology or simply to not perform experiments according to best practise in the field. We expect that seminars and personal training will increase the power of FACS based experiments at RWTH Aachen University.

2) Develop methods according to user demands. We see a particular need to a) standardize protocols for cell isolation from complex tissues and to establish staining panels that allow scientists/clinicians at RWTH to study cell population isolated from human tissues. b) step forward with respect to the analysis of FACS data. Conventional hierarchical gating strategies are more and more extended by new tool to analyse FACS data such as Boolean gating, bivariate difference gating and dimensionality reduction with the tSNE algorithms. We expect that FCF users will profit from a central platform to systemically build up these tools.

3) Set up regulations and instrumentation to handle primary human cells and S2 material. Currently the facility operates in an S1 environment and thus is not able to sort infected material or S2 material. Related to this issue, sorting of primary human material poses a notorious difficulty because of the potential infectious risk. It will be major aim to change this situation. To this purpose currently a DFG grant is prepared to purchase a cell sorter installed within a safety cabinet (see previous sections). Such instrument is critically needed to support the FACS based purification of potentially infectious material.

4) Expanded the FCF portfolio. New technologies such as image stream, CyTOF and spectral analysis are not yet available at RWTH Aachen University. FCF together with its users will define the most needed instruments and seek funding to extent instrumentation of the FCF.
Articles of Association

The Interdisciplinary Center for Clinical Research (IZKF) of the RWTH Aachen Faculty of Medicine

1. Preamble
The Interdisciplinary Center for Clinical Research (IZKF) is – pursuant to Section 70 (2) of the German University Act (HG), Section 10 University Hospital Regulations (UKVO) and Section 7 (1) of the articles of association of the university hospital – a funding program of the Faculty of Medicine of RWTH Aachen University for outstanding and strategically important research projects as well as for maintaining core facilities. For the fulfillment of its duties, the IZKF receives funds granted by the German State of North Rhine Westphalia (NRW) in accordance with the budget proviso in the budget of the competent ministry. The Dean's office decides on the amount of additional funding in coordination with the faculty board.

2. Purpose and objectives
The IZKF has developed and established structures and processes that continuously enable highly-qualified research at RWTH Aachen, incorporating the structural conditions of the Faculty of Medicine which offer a combination of health care services, research and teaching. The primary objective is the realization and funding of joint projects and individual projects with a thematic relevance to the core research areas of the Faculty of Medicine, as well as the funding of core facilities for the improvement of research opportunities.

2.1. The structural objectives are
- to further develop processes for promoting basic, translational and clinical research,
- to support the research profile of the university and faculty,
- to use funds pursuant to quality criteria,
- the transparent financing of research supplementary to the other programs of the faculty of Medicine
- the establishment and maintenance of core facilities.

2.2. The scientific objectives are
- the funding of research projects in accordance with the core research areas of the Faculty of Medicine,
- the planning, coordination and implementation of interdisciplinary research projects with the aim to achieve optimized research results through the joint use of the spatial, material and personnel resources,
- the encouragement of cooperation and information exchange between the Faculty of Medicine and the other faculties and institutions of RWTH Aachen,
- the assurance of scientific quality in the IZKF by means of internal and external evaluation.

3. Participants
3.1. The participants are the managers of the projects, core facilities (appointed by the steering committee) and research groups funded by the IZKF.

3.2. The participants commit
- to submit interim and final reports on the progress and results of their work to the steering committee in due compliance with predetermined deadlines,
- to reference the support of the IZKF in publications and presentations,
- to demonstrate appropriate involvement in public relations tasks.

3.3. The participation in the IZKF concludes
- upon termination of the employment agreement with the RWTH
- upon decision of the steering committee, if the relevant person severely impairs the activities of the IZKF or fails to meet his/her duties and obligations within the IZKF
- at request, to be communicated to the steering committee in writing.

4. The steering committee
4.1. The steering committee manages the IZKF and consists of the following members with voting rights:
- the speaker and deputy speaker, who are elected by the faculty board upon proposal by the dean for a period of three years. A re-election is possible.
- a research area coordinator and deputy research area coordinator from each faculty research area who may not be funded by the IZKF and is/are appointed by the relevant faculty research areas for three years. A re-election is possible. The deputy research area coordinator will be eligible to vote only in the absence of the research area coordinator.
- a representative of the core facilities, who is appointed by the core facility managers for a period of three years. A re-election is possible.
- a member of the natural science or engineering faculties of RWTH Aachen who is appointed by the faculty board for a period of three years upon proposal by the speaker. A re-election is possible.

The dean, the vice-dean for research as well as the speakers of the Collaborative Research Centers (SFBs), German Research Foundation (DFG) research groups and research training groups of the Faculty of Medicine and the research coordinator of the IZKF as well as the medical director and the commercial director can attend the meetings of the steering committee in an advisory capacity.

If a member of the steering committee resigns prior to the expiry of the three year period or if the research area coordinator belonging to the steering committee is funded by the IZKF, a re-election will be carried out in the faculty research area. If a faculty research area is not pursued further, the steering committee membership of the person coordinating the research area shall also be terminated.

4.2. The speaker represents the IZKF and manages the daily operations with the assistance of the IZKF administrative office. The speaker will submit an annual activity report to the Faculty of Medicine and the Rector of RWTH Aachen.
4.3. Tasks of the IZKF steering committee:

- overall responsibility for the IZKF including its further development,
- management of the business operations not included in daily operations,
- responsible for the distribution of the funds granted to the IZKF,
- decides within the scope of the available funds on the launch, termination and further funding of projects classified as eligible for funding,
- carries out internal quality controls,
- advises IZKF participants with regard to project-specific prospects,
- coordinates the use of the central laboratory space and core facilities allocated to the IZKF,
- approves the rules of procedure.

4.4. Research coordination

The steering committee is assisted by the research coordinator. He/she manages the business operations of the administrative office in a target and resource-oriented way in close cooperation with the administration of the university hospital. Within the scope of organisation development he/she adapts processes and develops an interdisciplinary personnel and process management under special consideration of quality aspects in accordance with the specifications of the steering committee.

5. Research funding

The following funding instruments are available:

- Joint projects: Joint projects are projects from several applicants working on one joint topic.
- Individual projects: Individual projects are projects with a maximum of two applicants on a fixed topic.
- Research groups: Research groups serve to support highly qualified junior scientists.
- Core facilities: Facilities in which equipment, expertise and methods are made available which are generally not accessible to individuals, but are nonetheless in the interest of the faculty. Core facilities are available to all members of the Faculty of Medicine.

6. Application

6.1. Eligibility

Eligible to apply are all post-doctoral scientists of the Faculty of Medicine who are employed at the UKA or RWTH on at least a part-time basis (50% of the regular working hours) and who can submit a declaration by the clinic or institute management, which is confirmed by the HR and Finance departments, that the financing of their position is guaranteed for the duration of the funding program. Project proposals by applicants who belong exclusively to other faculties of RWTH Aachen, may be admitted after prior request by the steering committee, if this is in the interest of the Faculty of Medicine. Project managers may not submit an application for their own position.

6.2. Procedure

The calls for proposal are published to all eligible applicants in accordance with 6.1 by the speaker of the steering committee in coordination with the dean of the Faculty of Medicine.

6.3. IZKF staff

The employment of IZKF-financed employees is applied for by the speaker upon proposal by the project manager for a period not exceeding the duration of the approved funding.

6.4. Termination of project funding

Project funding ends when the employment agreement of the person acting as project manager is terminated or interrupted, if no successor or interim arrangement has been determined by the steering committee of the IZKF. If in this event sub-projects should be carried out at a different site in order to successfully complete the project, this is possible for a fixed period of time. This course of action must be applied for at the steering committee by the new project manager. The funds released through the termination of a sub-project shall flow back to the IZKF. The investments in the relevant projects are subject to approval requirements of third-parties – owned by RWTH; the IZKF is entitled to these investments.

7. External experts

External experts are appointed by a work group consisting of the speaker, deputy speaker, dean, vice-dean for research and member of the steering committee representing the natural science and engineering faculties. In the case of a conflict of interests these can appoint additional work group members. External experts are reimbursed for their activities. Any incurred travel expenses will be refunded in accordance with the provisions of the travel allowance regulations.

External experts are responsible for the following tasks:

- They assess the quality of proposals and cast votes on the eligibility of the projects to receive funds. Negative votes are binding. Positive votes are to be implemented in accordance with the financial means of the IZKF.
- They evaluate research groups.
- They assess proposals of the core facilities for (follow-up) funding and give recommendations for increased funding, establishment or termination of core facilities.

8. Procedures

Unless special regulations have been agreed in these articles of association, the rules of procedure of the IZKF - as amended - shall apply should any procedural questions arise.

9. Amendments

If individual provisions of these articles of association should be invalid or amended by resolution of the faculty board of the Faculty of Medicine, this shall not affect the effectiveness of the remaining provisions.

10. Entry into force

These articles of association replace the version dated 6 May 2010. They come into force after being approved by the faculty board of the Faculty of Medicine and by the Rector of RWTH Aachen.

The articles of association dated 6 May 2010 apply to all projects in the current funding phase, ending on 30 June 2017. The regulations of the amended version shall exclusively apply for the new funding phase.