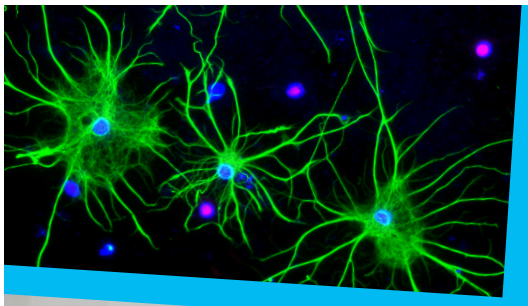
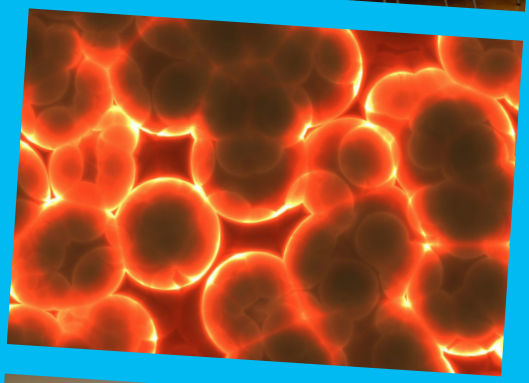
FRIEDRICH-ALEXANDER
UNIVERSITÄT
ERLANGEN-NÜRNBERG
MEDIZINISCHE FAKULTÄT

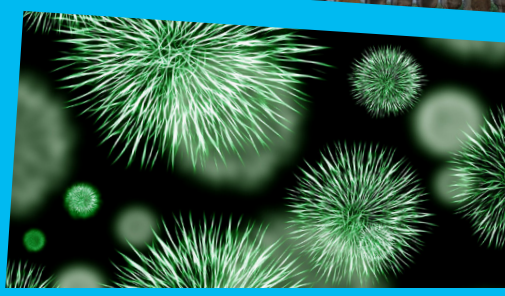
Universitätsklinikum
Erlangen

IZKF Erlangen
Annual Report 2019







interdisciplinary
Center for
Clinical Research

www.med.fau.de

IZKF Erlangen

Annual Report 2019

Editorial

In my position as the new Chairman of the Management Board, I am glad to present the Annual Report 2019 of the Interdisciplinary Center for Clinical Research Erlangen (IZKF), which represents the central structure of research development of the Faculty of Medicine of the Friedrich-Alexander-University Erlangen (FAU).

In compliance with our commitment to transparency, all IZKF progammes and ongoing IZKF-projects are presented in this annual report. Additionally, we have included an output analysis of previous years. If you are interested to learn more about the many activities the IZKF has to offer, you are invited to access and browse through our webpage www.izkf.med.fau.de.

This year has been marked by several developments, activities, but also by change. Such changes concern the composition of various IZKF-Committees:

Prof. Dr. Reis resigned from his position as Chairman of the Management Board. The IZKF sincerely thanks Prof. Reis for his tremendous efforts and strong dedication, his inspiration and initiatives over the past 12 years; otherwise the IZKF would not be where it is now. New members of the Management Board include Prof. Dr. Bozec as Deputy Chair of the IZKF, Prof. Dr. Mougiakakos, Prof. Dr. Schiffer and Prof. Dr. Neurath who has succeeded Prof. Dr. Dr. Schüttler as Dean of the Faculty of Medicine. A hearty welcome to all!

In 2019, the External Scientific Advisory Board experienced change as well. Prof. Dr. Sendtner finished his term as Chairman. His commitment and advice were exemplary. A big thank to Prof. Dr. Sendtner. He was succeeded by Prof. Dr. Seufferlein as the new Chairman of the External Scientific Advisory Board. Prof. Dr. Kuhlmann is the new Vice Chair. The IZKF is looking forward to many fruitful interactions.

The ELAN-Commission is now headed by Prof. Dr. Reis. At the same time four new colleagues joined the ELAN-Commission: Prof. Dr. Engel, Prof. Dr. Fejtova, Prof. Dr. Hellerbrand and Prof. Dr. Waldner.

Prof. Dr. Becker continues to run the Junior Scientists Committee. His new colleagues are Dr. David Dulin, Colin Griesbach, PD Dr. Janina Müller-Deile and Tatjana Seitz.

The IZKF supports research grants in all major research areas of the Faculty of Medicine. After three years, there was a new call for Advanced Projects last year. Prerequisites for a successful application to the IZKF were among others relevant preliminary work, first or last author publications in the recent past, own third-party funds and an interdisciplinary approach. On July 23 and 24, the internal colloquium for the preselection of projects for the new funding period took place. Out of 52 submitted project proposals, 46 projects were presented and 31 project leaders were invited to submit a full proposal by mid-September. The final evaluation by the External Scientific Advisory Board took place on November 14 and 15 in Erlangen.

On this occasion, the External Scientific Advisory Board attested the IZKF an outstanding scientific, structural and financial development and strongly recommended the continued provision of funds in the current amount of almost 6 million €, mostly from the Faculty of Medicine. The Board also commended the University for its continued commitment to co-funding including the allocation of funds from the emerging talents initiative ETI. Once again the IZKF has proven its aptitude as the central structure of research development of the Faculty of Medicine.

The External Scientific Advisory Board emphasized the high quality of the preselected proposals for Advanced Projects and recommended all 31 proposed projects for funding. The projects will start once personnel has been found for the approved positions, at the latest by July 1, 2020. As a rule, funding includes one staff position (doctoral student or technician) as well as consumables. If an application is submitted for external third-party follow-up funding on the topic of the project during the 30 months funding period, an extension of 6 months can be applied for. Tandem projects have the option of filling one of their positions at a later date without losing any of the approved financial resources for personnel.

During the reporting period, we witnessed again a high degree of successful research activity in the Junior Research Groups. They are an important instrument for attractive career development for outstanding young

scientists with training in medicine or life sciences. Over a period of up to 6 years each Junior Research Group receives generous funding for personnel and consumables and works independently in its own laboratories. Associations to individual clinics or institutes are possible, as are part time involvements in clinical activities for group leaders who are physicians. Currently, two Junior Research Groups are established under the leadership of Dr. P. Ceppi and Dr. D. Dulin. At the beginning of 2019, the junior research group of Dr. David Dulin on „Physics and Medicine“ moved with the Optical Imaging Center Erlangen OICE to its new building in Cauerstrasse. Dr. Dulin`s research group is now located in the Interdisciplinary Center for Nanostructured Films (IZNF) with access to an excellent infrastructure. Dr. Ceppi was offered a tenured position as Associate Professor in the Department of Biochemistry and Molecular Biology at the University of Southern Denmark in Odense. Dr. Ceppi accepted the appointment on a part-time basis while continuing his junior IZKF research group in Erlangen.

In addition to the call for Advanced Projects, there was also a call for Junior Projects in 2019. 14 applications from 10 different institutions were submitted until the March deadline. A selection colloquium took place on May 15 and 7 projects from 4 institutions were eventually chosen for funding. It is expected that physicians who receive Junior Project funding will complete the Clinician Scientist Programme (Advanced Module).

The Clinician Scientist Programme (CSP) is aimed at physicians who are in their specialist training, conduct their own research project and would like to continue their scientific education within the framework of a structured training programme. In 2019, numerous new participants from the Faculty of Medicine were accepted into the programme and the IZKF is pleased that the programme has been well received. In this context, we would also like to congratulate Dr. Zundler who is a junior project leader and participant in the Clinician Scientists Programme and received the Innovation Award of the German University Medicine (DHM). Dr. Zundler received the award for his research on a new immunotherapeutic approach to inflammatory bowel diseases (IBD) as recently published in Nature Immunology (PMID: 30692620).

Non-professorial group leaders and W2 professors without own budget strongly depend on extramural funding. Their research is particularly endangered by sudden unexpected loss or delay of funding. As life-saver in critical situations, we have established the bridging projects. The programme is devised as interim aid and is intended to allow personnel to be extended and the research project to be continued during funding gaps. The programme has been running only for a short time, but has already proven its value. A bridging project typically has a financial volume of €50T and duration of 6 months.

Of the many activities of the IZKF, I would like to highlight one event in particular. This is the International IZKF Symposium series. It is meant to strengthen the scientific exchange between researchers of the Faculty of Medicine. At the same time, it also presents an exceptional forum for internationally renowned speakers and serves to showcase IZKF-funded research of the Faculty of Medicine. This year we continued the series with the 7th International IZKF-Symposium. It took place on June 27 and 28 in Kloster Banz on the topic of “Translational Medicine” with a mixture of external and internal speakers in six sessions. On this occasion, we also awarded Publication and Poster Prizes and gave our awardees the opportunity to showcase their research in a short presentation. My gratitude goes to the organizing committee and to the speakers and sponsors for making this meeting possible. The next Symposium will be held from June 17-19 in 2021.

Last but not least, let me give you a short outlook on 2020: The submission deadline for the Junior Project proposals is going to be March 16, 2020, and the selection colloquium will be held on May 20, 2020. In parallel, we will also have a call for rotation positions within the Cinician Scientist Programme (Advanced Module).

Finally, I would like to thank you for your continued interest in and support of the IZKF. I also want to express my deepest gratitude to all the members of the Administrative Office who contributed once again to the success of the IZKF as a whole - and to the making of this annual report.

Prof. Dr. Michael Wegner
Chairman

Imprint and Contents

IMPRINT

Annual Report 2019

Publisher

Universitätsklinikum Erlangen
Interdisziplinäres Zentrum für Klinische Forschung (IZKF)
Chairman: Prof. Dr. Michael Wegner
www.izkf.med.fau.de

Editor

Dr. Katrin Faber
IZKF Administrative Office
Krankenhausstr. 12, 91054 Erlangen
phone: +49 9131 85 46841
fax: +49 9131 85 35903
katrin.faber@uk-erlangen.de

Cover

Anne Reichel
IZKF Administrative Office

Layout and setting

Bianca A. Meyerhöfer-Klee
IZKF Administrative Office

Photo credits

Cover + page 18: Bacteria by Pixabay

Portraits: The rights to portrait photographs are held by the Universitätsklinikum Erlangen and the IZKF Erlangen, respectively.

Printing

Druckhaus Haspel Erlangen e. K.
Willi-Grasser-Straße 13a, 91056 Erlangen

Print run

300 copies

April 2020

About us

Scientific Reports Advanced Projects

Scientific Reports Junior Groups/Projects

Scientific Reports Pilot Projects

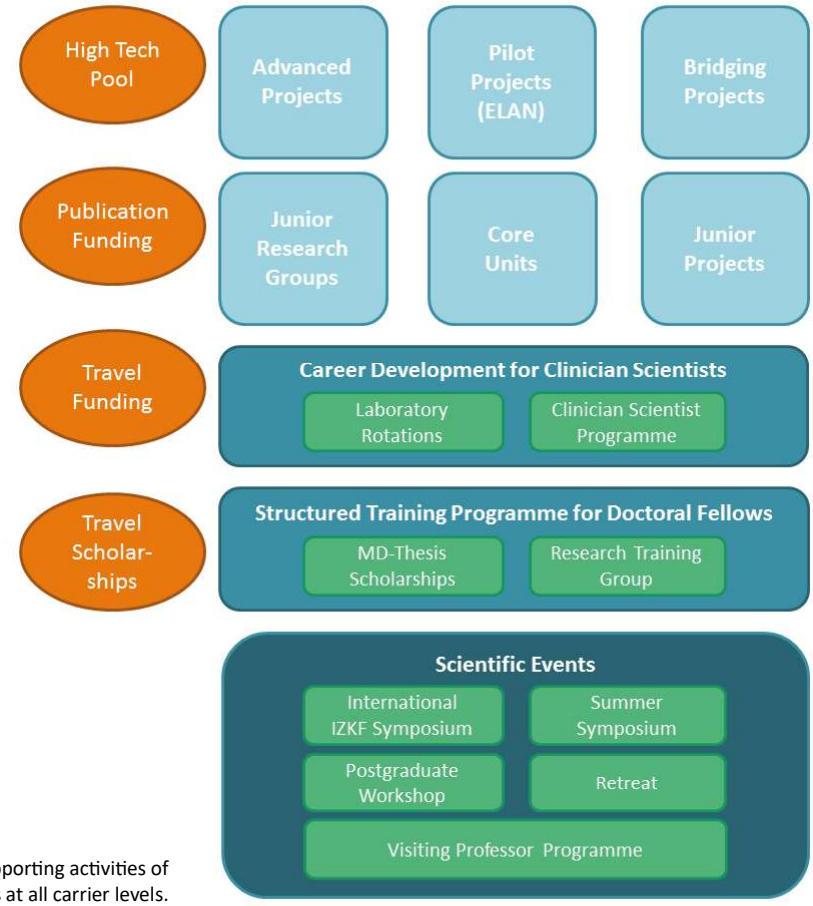
About us

Funding Schemes and Activities

Overview

The IZKF is the central structure of research development of the Faculty of Medicine. Its mission is to improve the overall quality of clinical research, to stimulate interdisciplinary research, to advance the careers of young scientists and to foster the acquisition of extramural funds. In order to achieve these goals, the IZKF supports projects in all research areas of the Faculty of Medicine on a strictly time-limited basis. The selection of projects is based exclusively on quality aspects. The various programmes are aimed at physicians and scientists at different stages of their scientific careers. Equipped with its own budget and own management structures, the IZKF continuously develops its own funding programmes in line with the needs of the Faculty of Medicine. In addition, the Faculty of Medicine also uses the structures established in the IZKF for the allocation and management of funds and avoids the creation of parallel structures.

The IZKF has created more transparency about research activities in the various areas and strengthened cooperation between clinics and institutes, but also between different clinics. The IZKF enables research funding beyond budget boundaries and also supports risk projects. Young scientists from Germany and abroad can be attracted by the junior research groups.



Programmes and supporting activities of the IZKF for scientists at all carrier levels.

Advanced projects

The IZKF offers research grants in all focal research areas of the Faculty of Medicine, i.e. immunology and infection research, renal and vascular research, neurosciences, tumor research and medical engineering. The project duration is 30 months. After a single funding period projects should be transferred to extramural funding. If the application for extramural funding was filed (as listed below) within the duration of the IZKF project, the duration of the projects will be extended for another 6 months.

LOM weighted 4-fold

- DFG
- BMBF
- Other Federal and State Ministries
- EU
- NIH-Grants

LOM weighted 2-fold

- Bayerisches Staatsministerium für Wissenschaft und Kunst
- Bayerische Forschungsstiftung/ Bayerische Landesstiftung
- Wilhelm Sander-Stiftung
- Volkswagen Stiftung
- Deutsche Stiftung für Herzforschung
- Humboldt-Stiftung
- Thyssen-Stiftung
- German-Israelian-Foundation (GIF)
- Mildred-Scheel-Stiftung/ Deutsche Krebshilfe
- Else Kröner Fresenius Stiftung
- José-Carreras-Stiftung
- Bill Gates Stiftung
- DAAD
- Deutsche Kinderkrebsstiftung/ HIT Deutsche Kinderkrebsstiftung
- Hertie-Stiftung
- Herman und Lilly Schilling-Stiftung

Funding agencies which allow the extension of projects according to the regulations of funds allocation in Bavaria (scientific performance criteria).

Single projects now include only one personnel position (graduate student or technical assistant). Applicants are expected to have an active publication record and own external funding. Preliminary results should yield the promise of a successful transfer of the project into external funding after the 30-months term. Innovative and original ideas and concepts are especially valued as well as the clinical relevance and interdisciplinary approaches. Applicants from all clinics, departments and institutes of the Faculty of Medicine and co-applicants from other faculties are entitled with no age limit.

Project funding is allocated after a stringent peer-review process based solely on scientific criteria. Research grants are approved after a two-stage review process. In an initial step, draft proposals are subject to an internal review by the Management Board, the Junior Scientists Committee, the ELAN-Commission and other recognized scientists of the Faculty of Medicine based on a written proposal and public presentation. Decisions are reached after internal deliberation and are communicated immediately afterwards. Successful proposals are presented in full to the Scientific Advisory Board during their peer-review site visits. Negative funding decisions of the Board are binding. Projects must start within six months after acceptance. Over the years funding rates were about 30 - 40%. Proposals are accepted every two or three years.

The last evaluation of the IZKF by the External Scientific Advisory Board took place on 14th/15th November 2019 in Erlangen. The high quality of the projects reviewed is demonstrated by the fact that the Board recommended all 31 projects that passed the internal review. Half of the projects (15) were rated as top projects, 11 projects received a very good rating from the Advisory Board and 5 projects received a good rating with funding recommendations, of which only 2 projects were reduced in terms of the requested funding.

The 42 new project leaders come from 23 different institutions. 12 (29%) of the project managers are women, 30 (71%) men. Project leaders include 19 (45%) natural scientists and 23 (55%) clinician scientists.

The projects will start with the filling of the approved positions, at the latest by 1 July 2020. One novelty is that tandem projects now have the option of filling their positions at a later date and thus do not lose any approved months of staff.

On the following pages you will find a list of all projects that were funded by the IZKF in 2019 and that will receive funding in the new funding period.

Staff	Single projects: graduate student or technical assistant (one position) Tandem projects: graduate student(s) and/or technical assistant (two positions)
Consumables	Single projects: EUR 15,000 p.a. Tandem projects: EUR 25,000 p.a.
Others	Participation in Travel, Publication and High Tech Pool
Duration	30 + 6 months

About us

Immunology and Infection

Project No.	Project title	Term	Applicant(s)	Institute
A63	Mechanisms of TNF-Mediated Control of Intracellular Pathogens in Mice and Man	01/07/2016-30/06/2019	Prof. Bogdan	Institute of Clinical Microbiology, Immunology and Hygiene
A64	The tyrosine-protein phosphatase SHP2 regulates TGFβ -dependent activation of JAK2/STAT3 in fibrotic diseases	01/02/2016-31/01/2019	Prof. Distler, Prof. Schett	Department of Medicine 3
A65	Tolerizing potential of human dendritic cell subpopulations	01/04/2016-31/03/2019	Prof. Dudziak	Department of Dermatology
A66	Genome wide CRISPR/Cas9 knockout for the identification of antiviral cellular restriction factors	01/07/2016-30/06/2019	Prof. Ensser	Institute of Clinical and Molecular Virology
A67	Analysis of the TRIM5alpha-mediated block to LINE-1 retroelements	01/02/2016-31/01/2019	Prof. Gramberg	Institute of Clinical and Molecular Virology
A68	Analysis of the role of the IL-23/Th17 axis during the control of antibody activity in rheumatoid arthritis	16/06/2016-15/06/2019	Prof. Krönke, Prof. Nimmerjahn	Department of Medicine 3, Division of Genetics
A69	Contribution of ATM kinase and the DNA-damage response in the innate immunity to infection	01/07/2016-30/06/2019	Prof. Lang	Institute of Clinical Microbiology, Immunology and Hygiene
A70	Novel targets for antiretroviral therapy - deubiquitinating enzymes regulate HIV-1 replication	01/07/2016-30/06/2019	Prof. Schubert	Institute of Clinical and Molecular Virology
A72	Targeted modulation of regulatory T cells and analyses of the underlying mechanisms	01/07/2016-30/06/2019	Prof. Steinkasserer	Department of Immune Modulation
A73	Checkpoint inhibitors as adjuvants for viral vaccines	01/07/2016-30/09/2019	Prof. Überla	Institute of Clinical and Molecular Virology
A74	The Role of Eosinophils in Allergic Bronchopulmonary Aspergillosis	01/06/2016-31/05/2019	Prof. Vöhringer, Prof. Krappmann	Department of Infection Biology, Institute of Clinical Microbiology, Immunology and Hygiene
A75	Role of MLKL-dependent programmed necrotic cell death in the pathogenesis of hepatitis	01/07/2016-30/06/2019	PD Dr. Dr. Günther, PD Dr. Dr. Wirtz	Department of Medicine 1
A76	Role of Gasdermin C in Gut Barrier Defence	01/02/2020-31/07/2022	Prof. Becker	Department of Medicine 1
A77	HIF expression in B cells regulates bone loss	30 months	Prof. Bozec	Department of Medicine 3
A78	Smurf2-IFN axis in IBD and mucosal healing	30 months	Dr. Dr. Chiriac, Prof. Neurath	Department of Medicine 1
A79	TR4 in tissue fibrosis	30 months	Prof. Distler	Department of Medicine 3
A80	Inflammasomes in primary dendritic cells	01/01/2020-30/06/2022	Prof. Dudziak	Department of Dermatology
A81	Receptor and neuropathogenicity of Bornavirus	01/01/2020-30/06/2022	Prof. Ensser	Department of Clinical and Molecular Virology
A82	Role of RANTES in the resolution of asthma	01/02/2020-31/07/2022	Prof. Finotto	Department of Medicine 1
A83	The role of SAMHD1 in CMV/ HIV coinfections	01/01/2020-30/06/2022	Prof. Gramberg	Department of Clinical and Molecular Virology
A84	Tissue-resident memory T cells in GvHD	01/06/2020-30/11/2022	Prof. Hildner, Dr. Zundler, Prof. Büttner-Herold	Department of Medicine 1, Department of Medicine 1, Department of Nephropathology

Project No.	Project title	Term	Applicant(s)	Institute
A85	The pathophysiology of SAPHO syndrome	30 months	Prof. Hüffmeier	Department of Human Genetics
A86	Characterization of synovial macrophage subsets	30 months	Prof. Krönke	Department of Medicine 3
A87	DC subsets and natural antibodies in leishmaniasis	30 months	Dr. Lehmann, PD Dr. Schleicher	Department of Dermatology, Institute of Clinical Microbiology, Immunology and Hygiene
A88	Cyclin interaction with a CDK-like viral kinase	01/02/2020-30/11/2022	Prof. Marschall, Prof. Sticht	Instiute of Clinical and Molecular Virology, Institute of Biochemistry
A89	CD83 regulates homeostasis and inflammation	30 months	Prof. Steinkasserer	Department of Immune Modulation
A90	The fate of lung-resident memory T-cells	01/01/2020-30/06/2022	Prof. Tenbusch	Instiute of Clinical and Molecular Virology
A91	Interfering with HTLV-1 persistence	16/03/2020-15/09/2022	Dr. Thoma-Kreß	Institute of Clinical and Molecular Virology
A92	FRCs and immune tolerance induction	01/02/2020-30/11/2022	Prof. Zaiss	Department of Medicine 3

Oncology

Project No.	Project title	Term	Applicant(s)	Institute
D24	Differentiation-associated Schwann cell transcription factors in melanoma - learning from embryogenesis	01/06/2016 - 31/05/2019	Prof. Dr. Bosserhoff, Prof. Dr. Wegner	Institute of Biochemistry
D25	Interaction of the EGFR- and the ZEB1-pathway in aggressive cancer types	01/05/2016 - 30/04/2019	Prof. Dr. Brabletz	Chair of Experimental Medicine I
D27	2-Hydroxyglutarate in Acute Myeloid Leukaemia: Novel Molecular Targets and Impact on Immune Escape	01/07/2016-30/06/2019	Prof. Mougiakakos	Department of Medicine 5
D28	SPARCL1 function in vessel maturation and metastasis of colorectal carcinoma	01/02/2016-31/01/2019	Prof. Stürzl, PD Dr. Naschberger	Department of Surgery
D30	Functional role of axin anchoring to microtubules in Wnt signaling	30 months	Prof. Behrens, Dr. Bernkopf	Chair of Experimental II
D31	Modulation of oncogene-induced senescence by cell-matrix adhesion	16/03/2020-15/09/2022	Prof. Bosserhoff	Institute of Biochemistry
D32	NPY in chemo-resistance and immune-escape in HCC	01/03/2020-31/08/2022	Dr. Dietrich	Department of Medicine 1
D33	Immunometabolism in CML	01/07/2020-30/06/2022	Prof. Metzler, Prof. Mougiakakos	Department of Pediatric and Adolescent Medicine
D34	Role of fibroblast polarization in the pathogenesis of colorectal carcinoma	30 months	PD Dr. Ramming, Prof. Stürzl	Department of Medicine 3, Department of Surgery
D35	Interactions of DPF3 and hypoxia in renal cancer	30 months	PD Dr. Schödel	Department of Medicine 4
D36	Endogenous retroviruses drive tumor inflammation	01/03/2020-31/08/2022	Prof. Strick, Prof. Hartmann	Department of Obstetrics and Gynecology, Department of Pathology

About us

Neurosciences

Project No.	Project title	Term	Applicant(s)	Institute
E19	Inhibitory neurotransmission in the cochlea: Glutamate and endocannabinoids	15/02/2016-14/02/2019	Prof. Enz	Institute of Biochemistry
E20	Identification of molecules, receptors and genes involved in chronic pruritus	01/05/2016-30/04/2019	PD Dr. Dr. Kremer, Prof. Zimmermann	Department of Medicine 1, Department of Anesthesiology
E21	Modulation of alpha-Synuclein pathology by FoxO-dependent pathways	01/05/2016-30/04/2019	Prof. Lie, Prof. Klucken	Institute of Biochemistry, Department of Molecular Neurology
E22	The role of Swiprosin-1/EFhd2 in resilience to drug addiction	01/03/2016-28/02/2019	Prof. Müller, Prof. Alzheimer, Prof. Mielenz	Department of Psychiatry and Psychotherapy, Institute of Physiology and Pathophysiology, Department of Molecular Immunology
E25	Modeling pain syndromes using human induced pluripotent stem cell-derived nociceptors	01/07/2016-30/06/2019	Prof. Winner, Prof. Schüttler	Department of Stem Cell Biology, Department of Anesthesiology
E26	Genetics and pathomechanisms of intellectual disability with microcephaly	01/03/2016-28/02/2019	Prof. Zweier	Institute of Human Genetics
E27	Lysophosphatidic acid-induced pruritus of cholestasis	01/03/2016-28/02/2019	PD Dr. Dr. Kremer, Prof. Fischer	Department of Medicine 1, Institute of Physiology and Pathophysiology
E28	Neural Crest Regulators In Orofacial Clefting	30 months	Prof. Gölz, Prof. Wegner	Department of Orthodontics and Orofacial Orthopaedics, Institute für Biochemistry
E29	The impact of lysosome dysfunction on stem cell ageing	01/01/2020-30/06/2022	Prof. Lie	Institute of Biochemistry
E30	Impact of the immune system on Parkinson‘s disease	01/04/2020-30/09/2022	Prof. Winner, Prof. Winkler	Department of Stem Cell Biology, Institute of Biochemistry
E31	Proteasomal degradation in intellectual disability	01/01/2020-30/06/2022	Prof. Zweier	Institute of Human Genetics

Renal and Vascular Research

Project No.	Project title	Term	Applicant(s)	Institute
F6	Renal afferent nerve activity - sympathoinhibitory or sympathoexcitatory?	01/07/2016-30/06/2019	Prof. Veelken, Prof. Amann	Department of Medicine 4, Department of Nephropathology
F7	Gpr126 (Adgrg6) in kidney development and disease	30 months	Prof. Engel	Department of Nephropatology
F8	Ion channel function of polycystin-2 in ADPKD	30 months	Prof. Korbmacher	Institute of Physiology
F9	Generation of novel glomerular 3D culture systems	30 months	PD Dr. Müller-Deile, Prof. Schiffer	Department of Medicine 4

The following table shows all institutions with a running Advanced Project in 2019 and their association to the focal research areas of the Faculty:

Institute	Main Research Areas			
	Immunology and Infection	Oncology	Neurosciences	Renal and Vascular Research
Chair of Experimental Medicine I		•		
Department of Anesthesiology			•	
Department of Dermatology	•			
Department of Immune Modulation	•			
Department of Infection Biology	•			
Department of Medicine 1	•	•	•	
Department of Medicine 3	•	•		
Department of Medicine 4				•
Department of Medicine 5		•		
Department of Molecular Immunology			•	
Department of Obstetrics and Gynaecology		•		
Department of Psychiatry and Psychotherapy			•	
Department of Stem Cell Biology			•	
Department of Surgery		•		
Division of Genetics	•			
Division of Molecular Neurology			•	
Division of Nephropathology				•
Institute of Biochemistry		•	•	
Institute of Clinical and Molecular Virology	•			
Institute of Clinical Microbiology, Immunology, and Hygiene	•			
Institute of Human Genetics			•	
Institute of Physiology and Pathophysiology			•	

About us

Pilot Projects (ELAN)

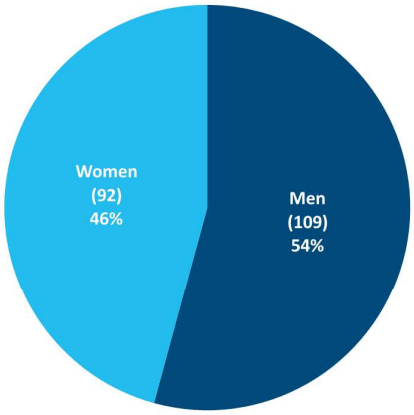
The aim of the ELAN programme is to support scientific projects at a very early stage and help prepare them for successful application for external funding (start-up projects), to support newly established working groups, to develop new innovative ideas (pilot projects) or act as interim funding if temporary gaps arise between individual extramural funding periods. Young scientists up to 38 years of age are supported with a maximum of € 60,000 for a period of up to 12 months. Since 2017, also applicants from the part of the Faculty administrated by the University have been approved for the programme, so that young scientists from the entire Faculty of Medicine can apply. In addition, newly appointed professors can submit their application regardless of age. A total of two ELAN projects can be applied for over the course of a scientific career, provided that a publication or a third-party funded project has arisen from the previous funding.

In 2017 the pilot project programme was newly integrated into IZKF. As a result, the nomenclature of the projects also changed. The projects now receive a P with a consecutive number as the project number. This replaces the old designation of the file numbers. Pilot projects are intended to support scientists at an early stage. In the reporting period of 2019, 32 proposals were discussed during the meetings of the ELAN-Commission. Thereof, 24 (75%) received funding. The approved projects cover nearly all the focal research areas of the Faculty of Medicine: oncology 7, immunology and infection 10, neurosciences 4, renal and vascular research 2 and medical engineering 1. In 2019, pilot project leaders from 19 different institutions were reviewed. In total, 15 (62%) of the successful applicants were men and 9 (38%) women. The median age was 33 for all project leaders.

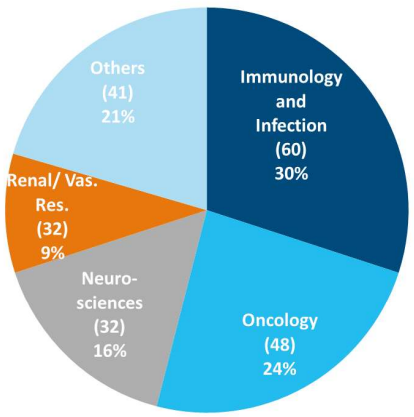
Applications for pilot projects can be submitted at any time. Since 2012 an electronic application using the ELAN-Tool is expected. The ELAN-Commission meets 5-6 times a year and selects projects for funding. The procedure provides for the participation of an external expert. Between 2012 and 2019 a total of 287 proposals for pilot projects were reviewed by the ELAN-Commission. Overall, 201 (70%) projects were granted for funding. Between 2012 and 2019 in total 92 women (46%) and 109 men (54%) applied successfully for pilot projects. The median age was at 34 years for all.

All focal research areas of the Faculty are represented; with immunology and infection (30%) and oncology (24%) being the most successful over the years.

Staff	One position
Consumables	max. EUR 20,000
Total funding	max. EUR 60,000
Others	Participation in Publication Pool and Travel Pool
Duration	max. 12 months



Gender distribution of pilot project leaders between 2012 and 2019.



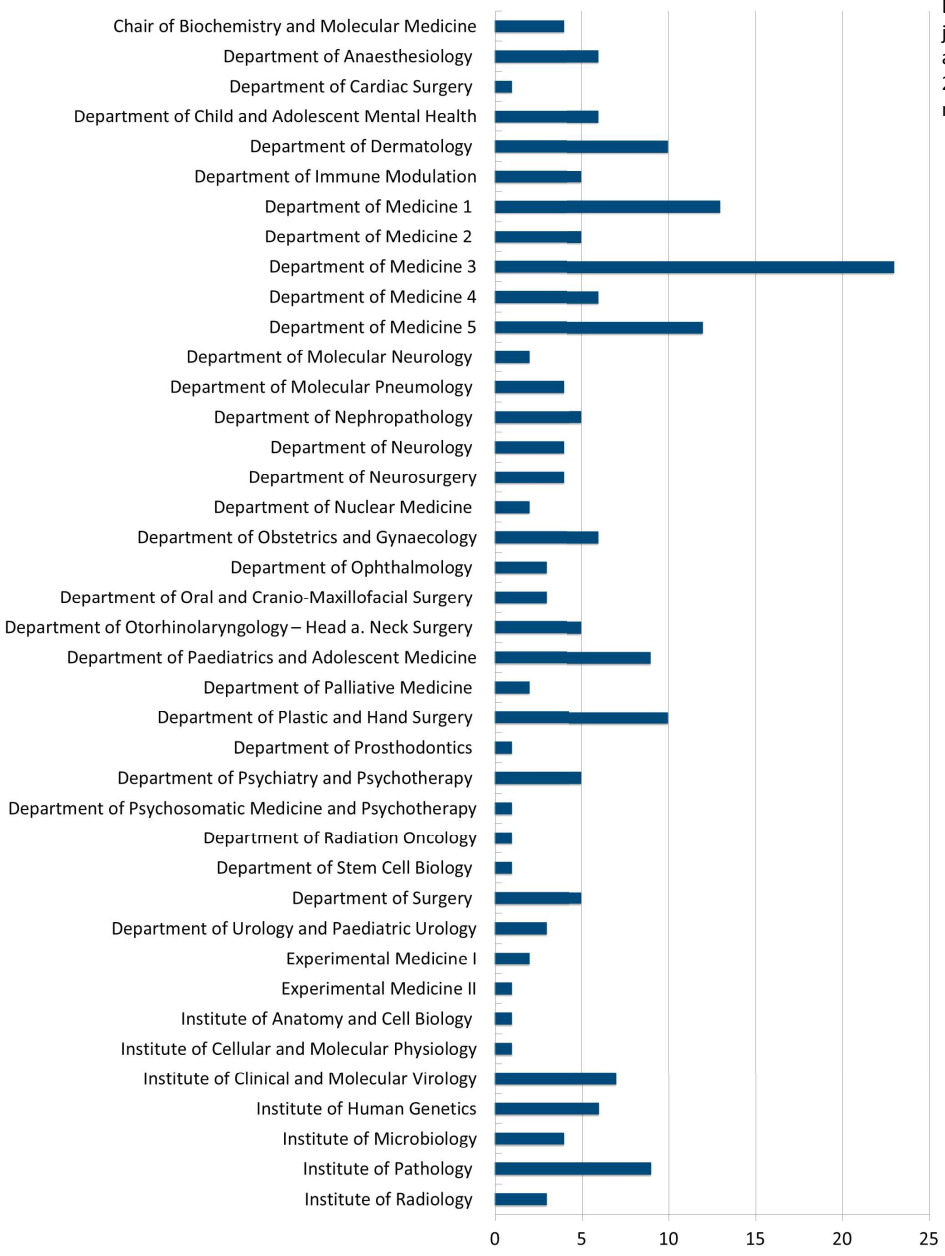
Distribution of pilot projects as per main research area between 2012 and 2019.

In the table all projects are shown which have received funding or have been approved for funding in 2019:

Project No.	Project title	Term	Applicant(s)	Institute
P016	Laboratory detection of ultrasound microbubbles	01/02/2018-31/01/2019	Dr. Knieling	Department of Pediatric and Adolescent Medicine
P018	XCR1 as a marker for human crosspresenting DCs	01/03/2018-28/02/2019	Dr. Heger	Department of Dermatology
P019	The involvement of the enteric nervous system in the immunopathogenesis of multiple sclerosis	01/06/2018-30/04/2019	Prof. Kürten	Institute of Anatomy
P020	Localisation of the EMT-transcription factor ZEB1	24/01/2018-23/01/2019	Dr. Eccles	Chair of Experimental Medicine I
P021	Bone strength in rheumatic finger joints	01/06/2018-30/04/2019	Dr. Kleyer	Department of Medicine 3
P022	Vascularization and bone formation	01/08/2018-31/07/2019	Dr. Steiner	Department of Plastic and Hand Surgery
P023	DPP4 - a molecular target in fibrosis	01/06/2018-31/05/2019	Dr. Soare	Department of Medicine 3
P024	The contribution of butyrophilins in the pathogenesis of Rheumatoid Arthritis	01/10/2018-31/03/2019	Dr. Sarter-Zaiss	Department of Medicine 3
P025	Analysis of exosomal biomarkers for CRSwNP	01/08/2018-31/07/2019	Dr. Müller	Department of Otorhinolaryngology - Head and Neck Surgery
P026	On myelination processes in the cuprizone model	15/11/2018-15/11/2019	Prof. Laun	Institute of Radiology
P027	IFN-gamma and vasculature in CRC	01/07/2018-31/12/2019	Dr. Britzen-Laurent	Department of Surgery
P028	3D organoid models for analysis of SOX11-CSS	01/10/2018-31/09/2019	Dr. Turan	Institute of Biochemistry
P029	Combined DNA-/RNA-transfection of T cells	01/11/2018-31/10/2019	Dr. Uslu	Department of Dermatology
P030	Ultrashort Echo Time MRI of Myelin at 7 T	01/01/2019-31/12/2019	Prof. Nagel	Institute of Radiology
P031	Zwicker tone as a model for acute tinnitus	16/12/2018-16/06/2019	Dr. Schilling	Department of Oto-Rhino-Laryngology - Head and Neck Surgery
P032	In vivo imaging of inflammation / bone remodeling	15/07/2018-14/07/2019	Dr. Schauer	Department of Medicine 3
P033	YB-1 in molecular regulation of chondrogenesis	26/03/2019-25/03/2020	Dr. Rottensteiner-Brandl	Institute of Biochemistry
P034	Myeloid ZEB1 in colorectal cancer	16/01/2019-15/01/2020	Dr. Schuhwerk	Chair of Experimental Medicine I
P035	Role of FBXO11 in intellectual disability	07/01/2019-07/01/2020	Dr. Gregor	Department of Humangenetic
P036	Physicians' opinions on continuous sedation	01/04/2019-31/03/2020	Dr. Heckel	Division of Palliative Medicine
P037	Prognostication in intracerebral hemorrhage	01/07/2019-30/06/2020	Dr. Sembill	Department of Neurology
P038	Molecular markers in stage T1 bladder cancer	01/04/2019-31/03/2020	Dr. Sikic	Department of Urology
P039	Bone characterization in early RA autoimmunity	01/06/2019-31/05/2020	Dr. Simon	Department of Medicine 3
P040	FoxO-dependent mitophagy in stem cell function	01/09/2019-31/08/2019	Dr. Schäffner	Institute of Biochemistry

About us

Project No.	Project title	Term	Applicant(s)	Institute
P041	Polyploid cardiomyocytes for cardiac repair	01/09/2019-31/08/2020	Dr. Leone	Department of Nephropathology
P042	Immunology of NMSC of the head and neck	01/09/2019-31/08/2020	Dr. Dr. Frohwitter	Department of Oral and Cranio-Maxillofacial Surgery
P043	The autotaxin-LPA axis in breast cancer	01/07/2019-24/08/2020	Dr. Kengelbach-Weigand	Department of Plastic and Hand Surgery
P044	HIF-1a in IgA class switching	16/08/2019-15/08/2020	Dr. Meng	Department of Medicine 3
P045	HSV-1 modulates the IL-6 signaling pathway in mDCs	16/06/2019-15/06/2020	Dr. Grosche	Division of Immune Modulation
P046	Magnetic Drug Targeting for head and neck cancer	01/11/2019-31/10/2020	Dr. Balk	Department of Oto-Rhino-Laryngology - Head and Neck Surgery
P047	Synaptic plasticity in the human hippocampus	16/06/2019-15/06/2020	Dr. Maslarova	Department of Neurosurgery
P048	Role of Collagen IV during retrovirus transmission	16/05/2019-15/03/2020	Dr. Thoma-Kreß	Institute of Clinical and Molecular Virology
P049	Inflammation from skin to joint	16/10/2019-15/10/2020	Dr. Raimondo	Department of Medicine 3
P050	Vascular, renal parameters in living kidney donors	01/01/2020-31/12/2020	Dr. Kannenkeril	Department of Medicine 4
P051	T follicular helper cells, transplantation	01/01/2020-31/12/2020	Dr. Spörl	Department of Medicine 5
P052	Treatment Response in EwS by PET/CT and ctDNA	01/10/2019-30/09/2020	Dr. Schmidkonz	Department of Nuclear Medicine
P053	The influence of serotonin on antigen presentation	01/10/2019-31/03/2020	Dr. Kretschmann	Department of Medicine 5
P054	Agrin and the neuromuscular junction in ALS	01/10/2019-30/09/2020	Dr. Krach	Deportment of Stem Cell Biology
P055	MSOT-imaging in spinal muscular atrophy	28/10/2019-27/10/2020	Dr. Regensburger	Department of Pediatrics and Adolescent Medicine
P056	Host-microbial interaction in the Liver	01/04/2020-31/03/2021	PD Dr. Dr. Günther	Department of Medicine 1
P057	Intravital microcopy in the AV-loop model	01/11/2019-31/10/2020	Dr. Hessenauer	Department of Plastic and Hand Surgery
P058	Wnt inhibitory peptide	12 months	Dr. Bernkopf	Chair of Experimental Medicine II - Molecular Oncology
P059	Regional neuroinflammation in RA	01/03/2020-28/02/2021	Dr. Süß	Department of Molecular Neurology
P060	ERVs in the tumorigenesis of MIBC	12 months	Dr. Eckstein	Department of Pathology
P061	Modified proteins in the gut break the tolerance and initiate autoimmune arthritis	12 months	Prof. Zaiss	Department of Medicine 3



Distribution of pilot projects among departments and institutes between 2012 and 2019 (absolute number of projects).

Bridging projects

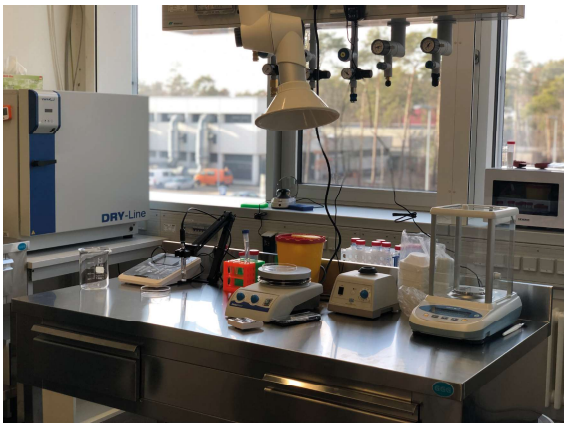
As of 2019, the programme will allow independent scientists (usually with a permanent employment contract) to work in the field of research to bridge a precarious situation, including professors from the part of the Faculty administrated by the University. The prerequisite is a recently rejected application for third-party funding which has

Staff and consumables	max. EUR 50,000 (the recruitment of new staff, especially of doctoral students is not intended)
Others	the use of central funds from the travel-, publication- and high-tech pool of the IZKF is not possible
Duration	about 6 months

About us

narrowly failed and which, after revision, can be submitted promptly. The application had to be filed to a donor, at least listed twice in LOM (scientific performance criteria), figure in the section Advanced Projects.

Other third-party- or intramural funding must not currently exist, but in the past a corresponding external funding (at least listed twice in LOM) must already have been available. A repeated use of the programme is only possible if the previous funding was successful, i.e. the resubmitted application for third-party funding was finally granted. Applications can be submitted at any time and promptly after the precarious situation has occurred. The amount of funding is up to € 50,000 for a period of 6 months. The evaluation is to be carried out by the ELAN-Commission in a circulation procedure. A member of the ELAN-Commission coordinates the evaluation and integrates a member of the External Scientific Advisory Board of the IZKF as an external expert. There is no age limit. The programme started recently.



Junior Research Groups

Junior research groups offer an attractive career development opportunity for outstanding young scientists with a training in medicine or natural sciences and a strong background and reputation in one of the Faculties' main research fields. Over a period of 6 years, each junior research group receives funding for the group leader, one postdoctoral scientist and one graduate student, one technical assistant and consumables. From this position several previous junior research group leaders have been appointed to a professorship or have achieved other attractive positions. The groups operate independently but may be associated to individual clinics or institutes. For physicians a part time involvement in clinical activities is possible. Groups also have access to research funds allocated by the Faculty based on scientific performance criteria (LOM). At the end of 2019 there are two junior research groups. One group (N1) is housed in the Nikolaus Fiebiger Center for Molecular Medicine with its attractive scientific environment and diverse activities; the other (N2) is located at the South-Campus in a new scientific building with the Optical Imaging Center Erlangen (OICE), where the group now has modern laboratories and offices with excellent equipment at its disposal.

Staff	Group leader Postdoctoral scientist Graduate student Technical assistant
Consumables	EUR 50,000 p.a.
Others	Participation in the allocation of funds based on performance criteria (LOM) Laboratory space Investment funds
Duration	6 years

Prof. Dr. Ceppi accepted a tenured position as Associate Professor in the Department of Biochemistry and Molecular Biology at the University of Southern Denmark in Odense while continuing his junior research group in Erlangen.

Project No.	Project title	Term	Group leader
N1	Understanding the plasticity of cancer cells	01/08/2015 - 31/07/2021	Prof. Ceppi
N2	Physics and Medicine	01/09/2016 - 31/08/2022	Dr. Dulin

Core Units

Modern molecular technologies, such as genomics, proteomics and advanced molecular imaging, require very expensive and complicated instrumentation and are methodologically very demanding. Thus it is often not scientifically worthwhile or cost-effective to establish and maintain these techniques in parallel in different groups. Core facilities or units are centralised methodological platforms that offer access to these modern methods and technologies to a broad user spectrum. This enables access to modern technologies to smaller groups and also to those with other main methodological interests. Also, it allows students to be directly exposed to these modern developments.

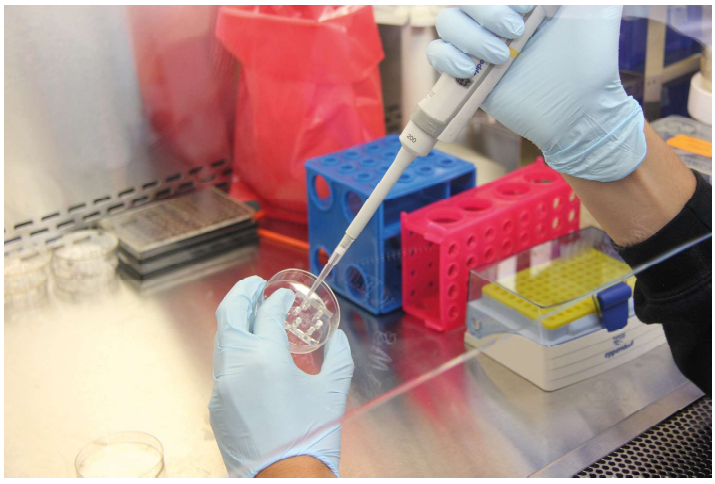
Core facilities are operated under the leadership of a scientific group with demonstrated excellence and interest in developing the methodology. In return for institutional support, it is expected that the operating group assists other groups with their know-how in accessing this technology. The support provided by the IZKF and the Faculty usually includes the initial investment for the instrumentation of the platform, the cost for setting up the operation as well as its continued technological development. IZKF pioneers the development of core facilities in Erlangen and usually supports them for an initial start-up phase of up to 6 years. Once established and successfully working, long-term support is provided directly by the Faculty.

Services and costs are to be made transparent and equal access has to be ensured. Core facilities are regularly evaluated for their effective operation, scientific excellence and timeliness.

The IZKF offers the possibility for developing new core units. Nearly all core units of the Faculty of Medicine are based on a start-up funding by the IZKF.

Next generation sequencing
Institute of Human Genetics, Prof. Dr. André Reis, Dr. Ekici
Cell sorting unit with immune monitoring
Dep. of Molecular Immunology, Prof. Dr. Hans-Martin Jäck Division of Genetics, Prof. Dr. Thomas Winkler Department of Medicine 5, Prof. Dr. Andreas Mackensen Dep. of Transfusion Medicine, Prof. Dr. Holger Hackstein Department of Dermatology, Prof. Dr. Carola Berking Sites: Nikolaus-Fiebiger Centre, Kussmaul Campus, Translational Research Center
Preclinical animal unit
Franz-Penzoldt Centre, Prof. Dr. Stephan von Hörsten
Small animal imaging - PIPE
Institute of Radiology, Prof. Dr. Michael Uder, Prof. Bäuerle

Core Units of the Faculty of Medicine currently in operation.

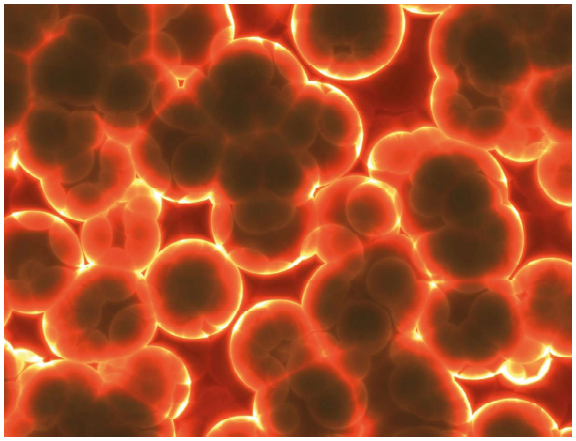


About us

Junior Projects

For scientists starting their independent career, obtaining their first extramural research funding is an important step. To aid in this process, the IZKF offers starting grants to young postdoctoral physicians and scientists up to 35 years of age without previous significant external funding. Candidates should have a visible publication record and projects should be based on an original idea with first tangible results.

Projects include a position for a technician or a graduate student and consumables for 30 months.



After this time it is expected that successful candidates submit an external grant application. If the application is filed within duration of the junior project, the spending period will be extended by another 6 months.

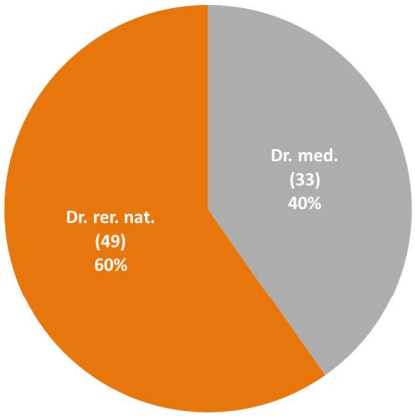
Junior projects are subject to a one-stage internal review only. Full proposals are reviewed by the Management Board, the Junior Scientists Committee and the ELAN-Commission based on a written proposal and public presentation. Decisions are reached after internal deliberation and are then communicated immediately afterwards to the proponents.

The first call for junior projects was in 2009. Proposals are accepted every year. Overall 82 junior projects were selected for funding between 2009 and 2019. In this period, 33 (40%) physicians received funding and 49 (60%) scientists. 21 (63%) of the physicians requested a laboratory rotation, thereof 7 (33%) were women and 14 (67%) men. Over the entire funding period, men and women were almost equally supported: 40 successful applicants were women and 42 men. The median age was at 32 at the time of application, for both women and men. All focal research areas of the Faculty are represented with immunology and infection (33%) and oncology (23%) being the most successful over the years. Overall candidates from 24 different institutions within the Faculty of Medicine were successful.

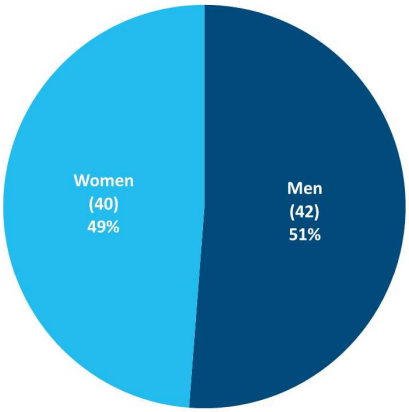
In 2019, 14 proposals were reviewed and 7 (50%) of them were selected for funding. The approved projects cover all of the focal research areas of the Faculty of Medicine. The successful applicants work in 4 different institutions within the Faculty of Medicine. In total, 1 (14%) is physician and 6 (86%) are scientists; 4 (57%) of the successful applicants are men and 3 (43%) are women. The median age was at 33 years.

Staff	Technical assistant or Graduate student
Consumables	EUR 15,000 p.a.
Others	Participation in Travel, Publication and High Tech Pool; IZKF laboratory rotations for physicians
Duration	30 months

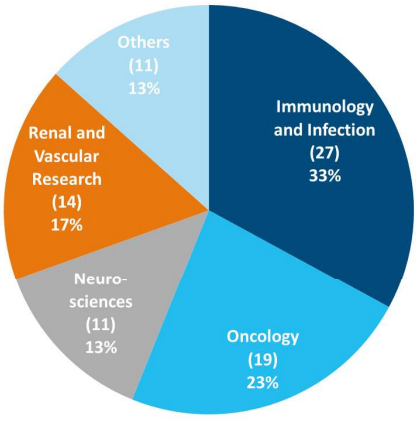
The following diagrammes show the distribution of junior projects per education, gender and main research areas over the period between 2009 and 2019.



Distribution of physicians (Dr. med.) and scientists (Dr. rer. nat.) between 2009 and 2019.



Gender distribution of junior projects between 2009 and 2019.



Distribution of junior projects as per main research area of the Faculty of Medicine between 2009 and 2019.

About us

In the following tables all junior projects are shown which received funding or were approved for funding in 2019.

Immunology and Infection

Project No.	Project title	Term	Applicant	Institute
J56	Epigenec reprogramming of macrophages	01/01/2017-30/06/2019	Dr. Palumbo-Zerr	Department of Medicine 3
J57	Herpesviruses and DUX4	01/01/2017-30/06/2019	Dr. Full	Institute of Clinical and Molecular Virology
J62	Mechanisms of neutrophil infiltration in rheumatoid arthritis	01/08/2017-31/01/2020	Dr. Grüneboom	Department of Medicine 3
J63	IL-3 in inflammatory bowel disease	01/12/2017-31/05/2020	Dr. Zundler	Department of Medicine 1
J69	Effect of HIV on pre-existing vaccine immunity	01/09/2018-28/02/2021	Dr. Nganou Makamdop	Institute of Clinical and Molecular Virology
J76	The role of itaconate-mediated metabolic reprogramming in osteoclasts	16/10/2019-15/04/2022	Dr. Kachler	Department of Medicine 3
J77	Phenotypic and Transcriptional Characterization of Auto-reactive B cells in Rheumatoid Arthritis	01/11/2019-30/04/2022	Dr. Pfeifle	Department of Medicine 3
J78	Role of GPX4-regulated ferroptosis in innate immunity to microbial infection	01/10/2019-31/03/2022	Dr. Ruder	Department of Medicine 1
J79	Functional impact of PD-L1:PD-1-interactions on diet-induced obesity and dysbiosis	01/09/2019-28/02/2022	Dr. Schwartz	Institute of Clinical Microbiology, Immunology and Hygiene

Oncology

Project No.	Project title	Term	Applicant	Institute
J58	Counteracting Wnt signaling	01/09/2016-28/02/2019	Dr. Bernkopf	Chair of Experimental Medicine II
J59	Immunotoxin induced anti-tumor immunity	01/07/2018-31/12/2019	Dr. Müller	Department of Medicine 5
J67	Metabolic reprogramming of AML MDSCs	01/01/2018-30/06/2020	PD Dr. Jitschin	Department of Medicine 5
J68	Role of GATA4 in Intestinal Inflammation & Cancer	01/10/2017-31/03/2020	Dr. Patankar	Department of Medicine 1
J73	Intracellular signaling by SPARCL1 in colon cancer	16/09/2018-15/03/2021	Dr. Tenkerian	Department of Surgery
J80	Psen1 in colorectal cancer	30 months	Dr. Mahapatro	Department of Medicine 1

Neurosciences

Project No.	Project title	Term	Applicant	Institute
J51	Inflammatory signature in Parkinson's disease	01/10/2015-31/03/2019	Dr. Marxreiter	Department of Molecular Neurology
J66	ß subunits: adding pieces to the puzzle of pain	01/01/2018-30/06/2020	Dr. Eberhardt	Department of Anaesthesiology

Renal and Vascular Research

Project No.	Project title	Term	Applicant	Institute
J64	Nephroprotection by HIF-hydroxylase inhibitors	01/10/2017-30/09/2018	Dr. Grampp	Department of Medicine 4
J65	T-System Regulation by Glucocorticoids	01/11/2017-30/04/2020	Dr. Seidel	Institute of Cellular and Molecular Physiology
J70	Gene discovery in kidney disease	01/10/2018-31/03/2021	Dr. Jobst-Schwan	Department of Medicine 4
J71	P2Y2R-dependent cyst growth in ADPKD	01/01/2019-30/06/2021	Dr. Kraus	Department of Medicine 4

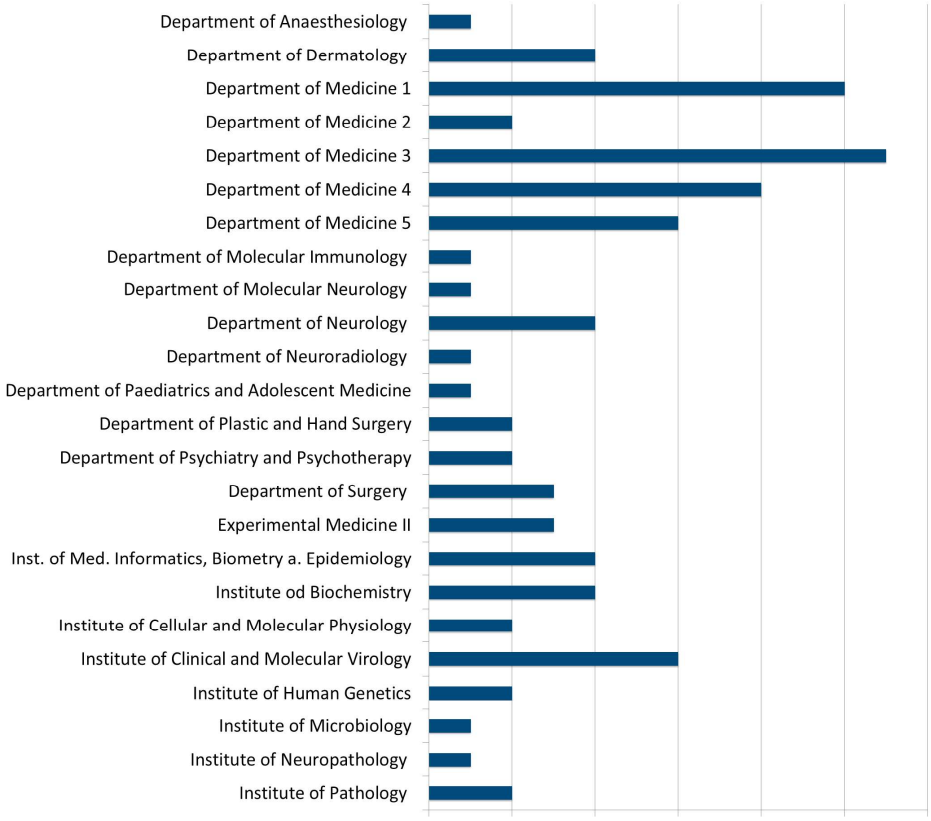
Molecular Medicine

Project No.	Project title	Term	Applicant	Institute
J74	The role of CtBP1 in hippocampal and cortical neuroplasticity	01/02/2019-31/07/2021	Dr. Salar	Department of Psychiatry and Psychotherapy
J82	O-GlcNAcylierung und Osteoklastogenese	01/01/2020-30/06/2022	Dr. Chen	Department of Medicine 3

Other methodologically oriented projects, informatics, statistics

Project No.	Project title	Term	Applicant	Institute
J61	Extending joint models in biomedical outcomes	01/01/2017-30/06/2019	Dr. Waldmann	Department of Medical Informatics, Biometry and Epidemiology
J75	Statistical Analysis of Infectious Disease Spread	16/10/2018-15/04/2021	Dr. Meyer	Department of Medical Informatics, Biometry and Epidemiology
J81	Web based Brain Tumor Image Classifier (WeB-TIC)	01/01/2020-30/06/2022	Dr. Jabari	Institute of Neuropatholgy

About us



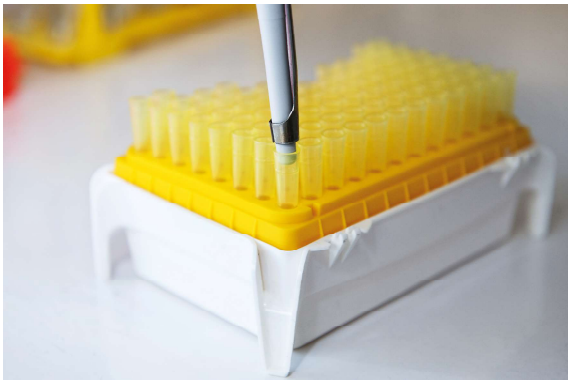
Distribution of junior projects among departments and institutes between 2009 and 2019.

Career Development for Clinician Scientists

Release from clinical work for research

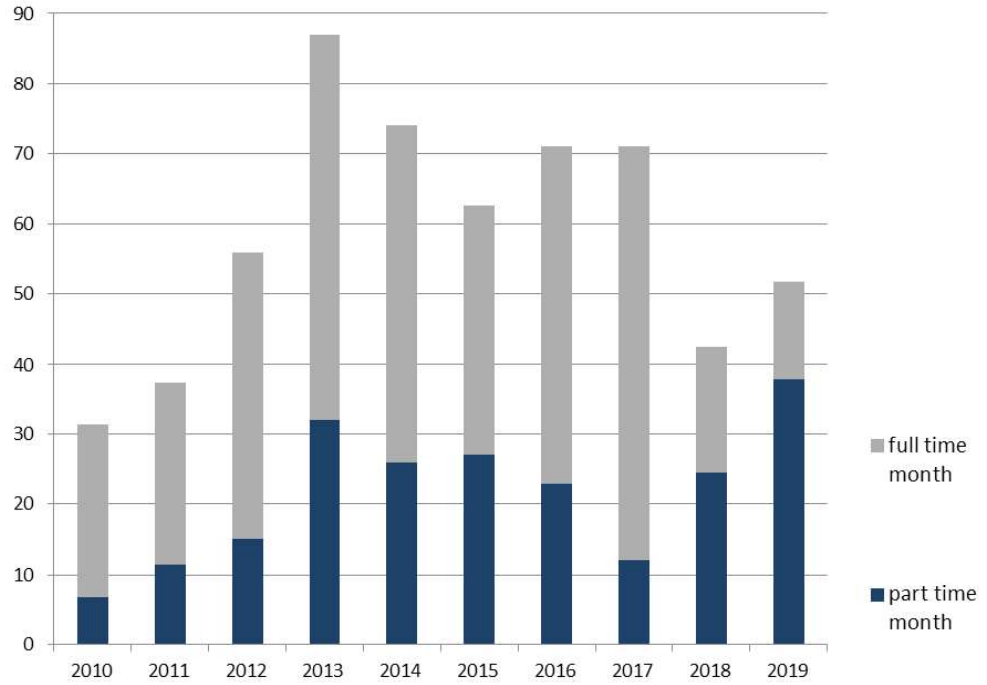
Access to protected research time is essential for young clinicians developing their projects. The laboratory rotation positions enable young scientists to fully devote themselves to a research project.

In the IZKF 6 rotation positions were financed continuously. Since 2017, the IZKF has been offering physicians who apply for a rotation position in the first applicant programme the opportunity to apply for a rotation position for 12 months full-time or 24 months part-time directly as part of the project application. In 2018, the first applicant programme and the rotation programme were expanded into a Clinician Scientist Programme for the entire Faculty of Medicine. Since then, 4 of the total of 6 rotation positions of the IZKF have been reserved for first-time applicants and applicants in the Clinician Scientist Programme. The other 2 rotation positions can still be used flexibly. The positions are available for a period of 6 months full-time or 12 months part-time, an extension is not (any longer) possible. This makes it possible to support up to 4 rotation projects per year. Applications may be submitted on an ongoing basis. There is no age limit, but the planned rotation position must make a suitable contribution to the scientific development of the respective applicant.



Name	Institution	Funding period	Rotating scope
Rotations			
PD Dr. Anita Kremer	Department of Medicine 5	10/2019 – 06/2020	100%
Dr. Stephanie Naas	Department of Medicine 4	05/2019 – 04/2020	50%
Dr. Florian Putz	Department of Radiation Oncology	09/2019 – 08/2020	50%
Dr. Adrian Regensburger	Department of Paediatrics	05/2019 – 10/2019	50%
Dr. Andrej Stoll	Department of Medicine 5	10/2019 – 06/2020	50%
Rotations of Junior Project Leaders			
Dr. Esther Eberhardt	Department of Anaesthesiology	07/2018 - 07/2019	50%
Dr. Steffen Grampp	Department of Medicine 4	04/2019 – 08/2020	50%
Dr. Tilman Jobst-Schwan	Department of Medicine 4	10/2018 – 09/2020	50%
Rotations of Clinician Scientists			
Dr. Franz Marxreiter	Department of Molecular Neurology	09/2018 – 05/2019, 07/2019 – 09/2019	100%
PD Dr. Andreas Kremer	Department of Medicine 1	01/2019 – 12/2020	50%
Dr. Markus Schüler	Department of Medicine 4	01/2019 – 12/2020	50%

Laboratory rotations running in 2019



The table shows the claimed months related to full time for each year. Due to the former lifespan of 12-24 months, the rotations usually last over a period of 2-3 calendar years.

About us

The Clinician Scientist Programme at the IZKF and the Medical Faculty

The Clinician Scientist Programme (CSP) is aimed at physicians who are in their specialist training, would like to conduct their own research project and to continue their scientific education within the framework of a structured training programme. Therefore, the CSP programme was launched at the end of 2018.

In 2016, the DFG hosted an event on „Clinician Scientists: Structured scientific qualification programmes for clinical researchers parallel to specialist training". 27 out of 37 medical faculties already have a CSP. An average of 20 participants takes part in the programme with a 6 or 12 months leave. The CSP includes professional as well as interdisciplinary further education, mentoring, retreats and monthly meetings. At the same time, the physicians conduct their own research project.

The aim of the CSP is to establish a new career path and promotion for Clinician Scientists and to create a structured scientific qualification programme for clinically researching physicians. The focus is also on strengthening translational research by creating time for scientific work and the preparation for habilitation.

The programme at the IZKF has a two-stage structure and is divided into a basic and an advanced module. The basic module lasts 2 years and requires a proof of the completed doctorate and the specialist training (already started at the time of joing the CSP).

The advanced module (duration 3 years) is aimed at physicians who have already successfully acquired a third-party funding, IZKF or first applicant project. The admission requirement for the advanced module is also fulfilled when having completed a post doctoral stay abroad of at least 2 years and at least 2 years of specialist training or with a successfully completed basic module. The leave of absence is 12 months full-time or equivalent part-time via rotation positions. An early change from the basic to the advanced module is possible on application under the following conditions: at least 2 years of specialist training and personally obtained IZKF- or third party funding. However, candidates who have been in the habilitation process for more than 2 years or who have already undergone an interim evaluation by the *Fachmentorat* cannot be accepted. In principle an early change into the advanced module is subject to a case-by-case examination and decision.

During the funding period, altogether 14 physicians took part in the CSP. At the same time as the application deadlines for the Junior Projects, a rotation position for participation in the CSP (Advanced Module) can be applied for at the IZKF on an annual basis. The following physicians participate in the Clinician Scientist Programme:

Basic Module

- Dr. Ingo Ganzleben, Department of Medicine 1
- Dr. Harriet Morf, Department of Medicine 3
- Dr. Stephanie Naas, Department of Medicine 4
- Dr. Adrian Regensburger, Department of Pediatrics and Adolescent Medicine
- Dr. Andrej Stoll, Department of Medicine 5
- Dr. Patrick Süß, Division of Molecular Neurology

Participants CSP 31.12.2019

Advanced Module

- Dr. Esther Eberhardt, Department of Anaesthesiology
- Dr. Steffen Grampp, Department of Medicine 4
- Dr. Ferdinand Knieling, Department of Pediatrics and Adolescent Medicine
- PD Dr. Andreas E. Kremer, Department of Medicine 1
- Dr. Franz Marxreiter, Division of Molecular Neurology
- Dr. Markus Schüler, Department of Medicine 4
- Dr. David Simon, Department of Medicine 3
- Dr. Sebastian Zundler, Department of Medicine 1

Structured Training Programmes for doctoral fellows at the IZKF

Life@FAU

In October 2017, the Graduate School for Life Sciences (Life@FAU) was launched following an initiative from the IZKF. It offers an interdisciplinary structured training programme for doctoral students at the Faculty of Medicine and the Department of Biology. The Faculty of Medicine and the Department of Biology at the Faculty of Sciences are involved on equal footing. All research training groups of both faculties are members of Life@FAU including the IZKF Research Training Group. The objectives of Life@FAU are to enhance structured training programmes for doctoral candidates at FAU, to create uniform standards in postgraduate education in the field of life sciences and to ensure the provision of structured training programmes. A Steering Committee with Prof. Christoph Becker (Department of Medicine 1) as chairman was elected.

Research Training Group	Registered participants
SFB 1181	36
GRK 2162	29
GRK 1962	16
TRR 130	9
GRK 1660	25
TRR 221	4
TRR 224	7
TRR 225	9
IZKF	76 + 53 MD
others	9
total	**273**

Research Training Groups participating in Life@FAU, indicating the number of participants (31/12/2019).

MD-Thesis Scholarships

This programme was initiated to arouse interest for science in motivated medical students early on in their career. Medical students are supported in performing an experimental thesis in association with the IZKF or externally funded projects. It is expected that they spend a significant time in a laboratory. Now up to 33 grants are available for medical students with outstanding performance and commitment in studies. The participants have to work full-time in the laboratory over a period of 9 months, whereby a scholarship is offered during their research activity of 8 continuous months. Furthermore, the doctoral students have to complete defined training modules during their studies. Training modules like guest speaker seminars, soft skill courses and the continuous supervision by a mentoring committee should continue throughout and until completion of the doctorate. Every participant of the MD-Thesis Scholarship Programme automatically becomes a member of the IZKF Research Training Group and the Graduate School of Life Sciences at FAU (Life@FAU) inaugurated in October 2017. Thus, the doctoral students can benefit from a structured, interdisciplinary training programme.

In 2019, a total of 30 medical doctoral students from 17 institutions were funded. Due to the fact that some scholarships granted in 2018 ended in 2019, the number of funded doctoral students is higher than the number of scholarships available. Overall, 30 applications for the MD-Thesis scholarship programme have been received in 2019. The Junior Scientists Committee approved 28 applications (93%), 13 (46%) of the successful applicants were females and 15 (54%) males. The median age was at 23 years.

Since its inception in 2007, the IZKF supported a total of 190 medical students with a scholarship. Medical students often initiate experimental work on their doctoral thesis during their studies. They will finish the thesis, though, only several years after they graduate. By the end of 2019, 58 (31%) students had already completed their doctoral thesis. Interestingly, 24 students (41%) obtained the highest degree possible, summa cum laude. This compares very favourably to the average 5% of all MD-Theses presented and is testimony to the excellent quality of MD-Theses performed within this programme.

About us

MD-Thesis Scholarships 2019

Department of Medicine 1
- Holle, Johannes Christian (05/2019 – 12/2019)
- Osterziel, Richard (05/2019 – 12/2019)
- Schürer, Hendrik (02/2019 – 09/2019)
- Stark, Markus (07/2018 – 02/2019)

Department of Medicine 3
- Koch, Julia (07/2019 – 02/2020)
- Schröder, Fenja (02/2019 – 09/2019)
- Schuster, Gregor (12/2019 – 07/2020)
- Thapa, Shreeya (05/2019 – 12/2019)

Department of Medicine 4
- Himmel, Lea (07/2019 – 02/2020)
- Nedoschill, Emmanuel (05/2019 – 12/2019)
- Schönau, Carlotta (05/2019 – 12/2019)
- Schwarz, Hannah (01/2019 – 08/2019)

Department of Medicine 5
- George, Rebekka (10/2019 – 05/2020)
- Noack, Rosa (12/2019 – 07/2020)
- Richter, Silja (10/2018 – 05/2019)

Institute of Biochemistry
- Grolig, Fabienne, (05/2019 – 12/2019)
- Lichtblau, Adrian, (06/2019 – 01/2020)
- Lüdje, Wichard, (03/2019 – 10/2019)
- Schneider, Marcel, (03/2019 – 10/2019)
- Vorhauer, Lisa, (03/2019 – 10/2019)

Others
- Auth, Janina, **Institute of Clinical and Molecular Virology** (12/2019 – 07/2020)
- Breakell, Thomas, **Institute of Anatomy** (02/2019 – 09/2019)
- Füermann, Florian, **Department of Psychiatry and Psychotherapy** (05/2019 – 12/2019)
- German, Alexander, **Institute of Radiology** (05/2019 – 12/2019)
- Gregoric, Gaspar, **Institute of Radiology** (09/2019 – 04/2020)
- Kapp, Michael Chr., **Institute of Microbiology** (07/2019 – 02/2020)
- Kuczera, Lukas, **Division of Molecular and Experimental Surgery** (01/2019 – 08/2019)
- Lambrecht, Vera, **Division of Molecular Neurology** (01/2019 – 08/2019)
- Lanfer, Jonas, **Department of Stem Cell Biology** (02/2019 – 09/2019)
- Neußel, Gregor, **Department of Anesthesiology** (02/2019 – 09/2019)
- Ostendorf, David, **Department of Plastic and Hand Surgery** (01/2019 – 08/2019)
- Rau, Ludwig, **Chair of Experimental Medicine I** (10/2018 – 05/2019)
- Ronicke, Moritz, **Department of Dermatology** (10/2018 – 05/2019)
- Schmitz, Deborah, **Department of Plastic and Hand Surgery** (01/2019 – 08/2019)
- Volz, Christian, **Department of Pediatrics and Adolescent Medicine** (05/2019 – 12/2019)

Research Training Group

The IZKF runs a research training group for all doctoral fellows and MD-students of the IZKF. Participation is mandatory for all doctoral candidates in sciences who are not involved in an alternative structured training programme of the Faculty/ University and for doctoral candidates who receive funding as part of an IZKF MD-Thesis scholarship. Other students may also associate with the research training group.

Aims of the IZKF Research Training Group include fostering networking and scientific self-organisation, methodological competence and soft skills as well as offering insights into other scientific fields and career opportunities. A structured seminar programme, courses in basic methods, in scientific writing and presentation are organised by the IZKF. In addition, the participants of the graduate school have to attend guest speaker seminars and to participate in the annual internal retreat. Participation in external congresses and in seminars organised by the doctoral students are mandatory.

The research training group also offers a mentoring programme for all doctoral students. Each doctoral student announces three mentors. At least one annual meeting of the doctoral student and the mentoring committee is expected.

The IZKF Research Training Group is divided into four research areas: Jour Fixe Ink (immunology/infection/ renal and vascular research), Jour Fixe Neuro (neuroscience), Jour Fixe Onko (Oncology) and the Jour Fixe DigIT.

Course “Intellectual Property Rights” given on 24th May 2018.

Courses given in 2019

The following soft skill- and statistic courses were given in 2019:

- Scientific Writing 1 An introduction to scientific writing: Dr. Deborah Bennett, 3rd-5th July 2019
- Scientific Writing 2 Writing research articles: Dr. Deborah Bennett, 29th July-2nd August 2019
- Scientific Writing 3 Writing a Phd Thesis: Dr. Deborah Bennett, 2nd-6th September 2019, 11th-13th December 2019
- Presentation Skills: Dr. Deborah Bennett, 3rd-5th April 2019, 24th – 26th July 2019
- Intellectual Property Rights: Prof. Dr. Christian Pilarsky, 5th July 2019
- Biostatistics: Dr. Matthias Englbrecht, 28th-29th March 2019
- Project Management Basics: Axel L. Lechler, 2nd April 2019

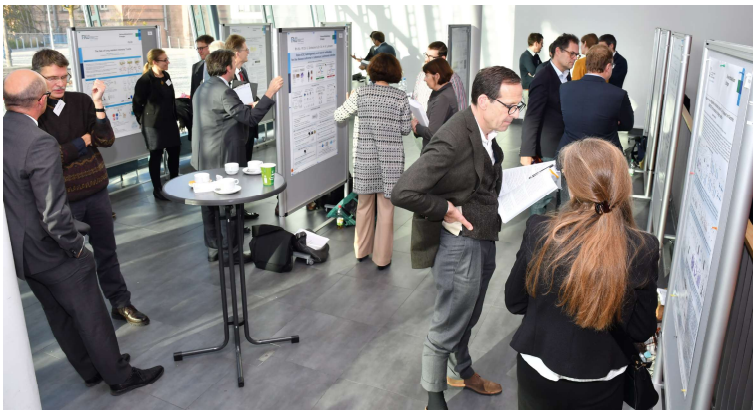
About us

Events of the IZKF Erlangen

IZKF - Assessment 2019

The evaluation of the IZKF by the External Scientific Advisory Board took place on 14th/15th November 2019 in Erlangen. The External Scientific Advisory Board certifies the IZKF an outstanding scientific, structural and financial development and strongly supports the continuation of the funds in the amount of almost 6 million €. The Board is also pleased with the extension of the University's funding commitment of almost 400,000 € including ETI-funding. The high quality of the projects reviewed is demonstrated by the fact that the Board recommended all 31 proposed projects for funding. Half of the projects (15) were rated as top projects, 11 projects received a very good and 5 projects a good rating with a funding recommendation, of which only 2 projects were cut back in terms of funding. The projects will start with the filling of the approved positions, at the latest by 1st July 2020. One novelty is that tandem projects now have the option of filling their positions at a later date and thus do not lose any approved months of staff. All core projects were recommended for further funding in the amount applied for. The IZKF expressly thanks the members of the External Scientific Advisory Board for their visit, their intensive involvement with the projects and the IZKF and for the numerous valuable recommendations. Our special thanks go to the outgoing Chairman of the Board, Prof. Sendtner. The members of the Board elected Prof. Seufferlein from Ulm as the new Chairman and Prof. Tanja Kuhlmann from Münster as his deputy. However, Prof. Sendtner remains a member of the External Scientific Advisory Board until the end of the year. The IZKF is looking forward to the continuation of the trusting and successful cooperation with the Board.

Kick-off event of IZKF-Assessment on November 14, 2020



Poster session during the IZKF-Assessment on November 14, 2020

International IZKF Symposium Kloster Banz

The IZKF regularly organises international scientific symposia which are held at the conference center at the baroque monastery of Kloster Banz in the upper Main valley. This venue offers a unique stimulating and interactive environment. An attractive programme with many speakers from Germany and abroad is developed by a Programme Committee. In addition, the participants of the symposium present their concepts and results in poster sessions. All interested scientists are welcome to join the symposium.

Conference hall at the IZKF Symposium

In 2019, from June 27 to 28, the 7th International IZKF-Symposium took place on the topic of „Translational Medicine". The proven concept of sessions with 2 external and 2 FAU speakers was again implemented. Next to this, the Publication Award and Poster Prize Winners gave a short presentation on the last conference day.

Session I: Medical Technology

Session II: From Cells to Organoids

Session III: Personalised Medicine

Session IV: Poster Session

Session V: Immunotherapy and Prevention

Session VI: Omics and Big Data for Precision Medicine

This year's winner of the Publication Award is **Simon Rauber** on the subject of "Resolution of inflammation by interleukin-9-producing type 2 innate lymphoid cells".

The Poster Prizes went to:

- **Kristina Scheibe** „Inhibiting Interleukin 36 Receptor Signaling Reduces Fibrosis in Chronic Intestinal Inflamm ation",
- **Annemarie Schwab** „Role of Polyol Pathway in Lung Tumorigenesis",
- **Julia Straub** „Aberrant glycosylation of the interferon gamma receptor alpha chain inhibits the response to IFN-γ in colorectal carcinoma cells" and
- **Iris Schäffner** „FoxO-dependent control of lysosome function in adult neurogenesis".

From the left: Prof. C. Becker, I. Schäffner, A. Schwag, S. Rauber, J. Straub, K. Scheibe, Prof. A. Reis

The next Symposium will take place on **June 17 and 18 in 2021.**

About us

Impression from the IZKF Postgraduate Workshop 2018.

Postgraduate Workshop/Hörsaalzentrum

Every two years, the Junior Scientists Committee organises the IZKF Postgraduate Workshop. The Postgraduate Workshop alternates with the IZKF International Symposium at Kloster Banz. At the IZKF Postgraduate Workshop, lectures are held by internationally recognized speakers on a timely topic. The focus of the workshop is on a poster session in which all members of the graduate school are expected to present their projects. Two poster prizes are awarded.

On Wednesday, 17th October 2018, the IZKF Postgraduate Workshop took place. During the poster session in the foyer, the doctoral students of the IZKF Graduate School presented their scientific work with a poster. The three best posters were awarded a prize by the Junior Scientists Committee. The next Postgraduate Workshop will take place on 6th October 2020.

Retreat

In 2019 the second IZKF-Retreat took place at the Fraunhofer Research Campus in Waischenfeld on 1st and 2nd October. Within five sessions, 44 medical- and scientific doctoral students from the IZKF but also from associated projects were able to present their field of research to the other participants. All the lectures lasted 15 minutes, followed by a brief discussion. This year's topics were heart, liver, gut (session I), therapy and oncology (session II), neurology (session III), immune cells and microenvironment (session IV) and infections (session V). The projects were presented during the two days and various methods and questions were discussed in round table sessions.

Walk & Talk Session of the IZKF-Retreat

The key note was held by Professor Benz from Munich, who inspired the participants in a lecture about his research and personal career.

On the evening of the first day the documentation „Paywall: The Business of Scholarship“ (documentary which focuses on the need for open access to research and science) was shown. Time for exchange and getting to know each other was offered by the Walk & Talk session.

The next IZKF Retreat is planned for 2020.

Special Programmes

Special programmes provide additional funding for IZKF projects but not all programmes are available to all funding lines of IZKF.

High Tech Pool

The IZKF actively encourages the use of modern "omics" technologies in the projects, such as those provided by the Core Unit Next Generation Sequencing. Since these experiments are generally expensive and consumables within IZKF advanced and junior projects are restricted, additional support is necessary. Costs for consumables can therefore be supported upon request with up to € 10,000 per project, provided that the project itself contributes at least 30% of the total sum, if the total exceeds € 5,000. This programme is available for advanced and junior projects but not for ELAN- and bridging projects.

Travel Funding

To enable IZKF members to present their results to the academic community, the IZKF supports their participation in international conferences. All applicants are expected to give a lecture or present a poster. The subject matter of the event must be related to the IZKF project in order to receive funding. The financial contribution of the IZKF is limited to € 500 for conferences in Germany, € 1,000 in Europe, and up to € 1,500 for conferences outside Europe.

This programme is also available for successful applicants for MD-Thesis scholarships and laboratory rotations (up to 6 months after the end of the funding period), but not for pilot projects.

Publication Funding

The publication of results obtained in IZKF projects in scientific journals is actively supported. It is expected that the IZKF funding of the project is acknowledged. The IZKF covers publication costs up to € 1,500. If the total costs are more than € 3,000 a financial contribution of € 2,000 is given by the IZKF. This programme is also available for successful applicants for MD-Thesis scholarships, laboratory rotations and pilot projects (up to 12 months after the end of the funding period, respectively).

Travel Scholarships

Travel scholarships allow IZKF's young researchers to spend time at other laboratories in Germany or abroad to conduct important experiments or learn the latest techniques and methods. The programme also allows doctoral candidates to intensify existing collaborations or establish new ones. Travel grants include transportation and accommodation for up to 3 months. An extension of the travel scholarship for another 3 months is possible.

Visiting Professor Programme

To encourage cooperation and to foster the exchange of ideas, IZKF promotes visits of external scientists. Currently it administers and supports two complementary programmes.

IZKF Visiting Professor Programme

The IZKF Visiting Professor Programme has been running successfully for many years. Every year approx. 10 scientists from abroad but also from other places in Germany can be invited for a stay of between 2 days and 4 months. The programme covers an amount of up to € 3,000 for travel and accommodation costs for visiting researchers. Application is restricted to IZKF members and the invited researcher's subject must be related to IZKF. Since the existence of the FAU Visiting Professor Programme the IZKF Programme is focused on promoting younger scientists.

FAU Visiting Professor Programme

IZKF manages the FAU Visiting Professor Programme according to the FAU bylaws. The university supports visits of professors from abroad and with high international reputation with fixed travel and day allowances. At least one presentation must be given in Erlangen, with members of the Faculty being invited. It is currently still open whether the programme can be continued within the existing framework for the Faculty of Medicine.

About us

The special programmes of the IZKF offer an additional financing for projects funded by IZKF. They are available for the following funding lines:

	High Tech Pool	Travel Pool	Publication Pool
Advanced Projects (Project leaders and scientific staff financed by project)	✓	✓	✓
Junior Projects (Project leaders and scientific staff financed by project)	✓	✓	✓
Pilot Projects (Project leaders and scientific staff financed by project)	✗	✓	✓
Bridging Projects	✗	✗	✗
Junior Research Groups (Project leaders and scientific staff financed by project)	✓	✓	✓
Clinician Scientists Programm (IZKF laboratory rotations)	✗	✓	✓
Clinician Scientists Programme (other funding)	✗	✗	✗
Other IZKF laboratory rotations	✗	✓	✓
MD-Thesis Scholarships	✗	✓	✓
Time frame	only within project period	6 month after the end of the project (MD: 12 month after the end of the scholarship)	12 month after the end of the project
The **Travel Scholarships** are available for young scientists (project leaders of Junior Projects, participants CSP, participants IZKF-laboratory rotation, doctoral students of all IZKF projects) only within the project period.			

Scientist, Institute	Visit	Lecture title
Prof. Dr. Shirani Ranasinghe, Peradeniya, Sri Lanka Department of Biochemistry/Sri Lanka	March 2019	Impact of mineral homeostasis on the pathogenesis of some common diseases in Sri Lanka
Prof. Dr. Gilbert Möckel, Yale School of Medicine, CT/USA	December 2019	MIF-2/D-DT is a cytokine with cell protective and regenerative function in the kidney proximal tubule

IZKF Visiting Professor Programme

Scientist, Institute	Visit	Lecture title
Prof. Dr. Hans Schreiber, Cancer Research Institute, Chicago/USA	March 2019	T-cell based immunotherapy and the tumor microenvironment
Dr. Saar Gill, Philadelphia/USA	March 2019	Genetic inactivation of CD33 in hematopoietic stem cells to enable CAR T cell immunotherapy for AML
Prof. Dr. Carl June, Pennsylvania Perelman School of Medicine, Philadelphia/USA	March 2019	Next generation CAR T cells for lymphoma/ myeloma
Dr. Gioele La Manno, EPFL/ Switzerland	June 2019	Dissecting brain function using single-cell transcriptomics
Dr. Ramanuj DasGupta, GIS/ Singapore	June 2019	Phenotype-driven precision oncology - guiding treatment "one patient at-a-time"
Prof. Dr. Nils J. Faergemann, University of Southern Denmark/Denmark	June 2019	From metabolomics towards in situ fluxomics in clinical sciences
Prof. Dr. Zlatko Trajanoski, Medizinische Universität Innsbruck/Austria	June 2019	Perturbation biology for informing precision immuno-oncology
Prof. Dr. Christian Blank, NKI Amsterdam/ the Netherlands	June 2019	Neoadjuvant checkpoint inhibition in melanoma - the template for personalized immunotherapy
Dr. Amir Fattahi, Tabriz University of Medical Sciences, Tabriz/ Iran	July 2019	Prostaglandins, Wnt, TGF-β, and Notch signaling pathways in uterus of mice mated with seminal vesicle-excised males during window of implantation
Prof. Dr. Katharina Brandl, University of California, San Diego/La Jolla/USA	July 2019	Apps and more: How to put your students' phones into good use
Dr. Mohammad Javed Ali, University of Rochester, New York/USA	September 2019	Recent fascinating advances in lacrimal sciences
Dr. Hugo Vicente Miranda, NOVA Medical School/ Universidade Nova de Lisboa/Portugal	September 2019	Lessons from Diabetes: Novel Parkinson's disease causative factors
Prof. Dr. Nicole Li-Jessen, McGill University, Montreal/Canada	December 2019	Towards a bioinstructive design of scaffold biomaterials for vocal fold tissue engineering

FAU Visiting Professor Programme

About us

Governance and Statistics

The IZKF in Numbers

24 Advanced Projects

12 Immunology and Infection
4 Oncology
7 Neurosciences
1 Renal and Vascular Research

7 tandem projects between departments and institutes
24 projects completed in 2019
35 project leaders

2 Junior Research Groups

34 Institutions with running projects 2019

23 Junior Projects

9 Immunology and Infection
5 Oncology
2 Neurosciences
2 Renal and Vascular Research
3 Others
thereof **6** projects completed in 2019

39 Pilot Projects

24 Newly granted in 2019
16 Projects completed in 2019
6 Projects approved in 2019, starting 2020

84 Employees of the IZKF

55 Doctoral fellows, Post-Docs and laboratory rotations
29 Non-scientists

1 patents

78 Ongoing Scientific Theses in 2019

10 Master theses
53 Doctoral theses
4 Habilitations
11 Laboratory rotations

11 awards

3 Appointments of IZKF project leaders to W2/ W3 - positions

273 Members of Life@FAU 2019

36 SFB 1181
29 GRK 2162
16 GRK 1962
9 TRR 130
25 GRK 1660
4 TRR 221
7 TRR 225
129 IZKF
53 Dr. med.
76 Dr. rer. nat.
9 participants outside RTG

4,374 K€ total expenditures in 2019

43 Publications

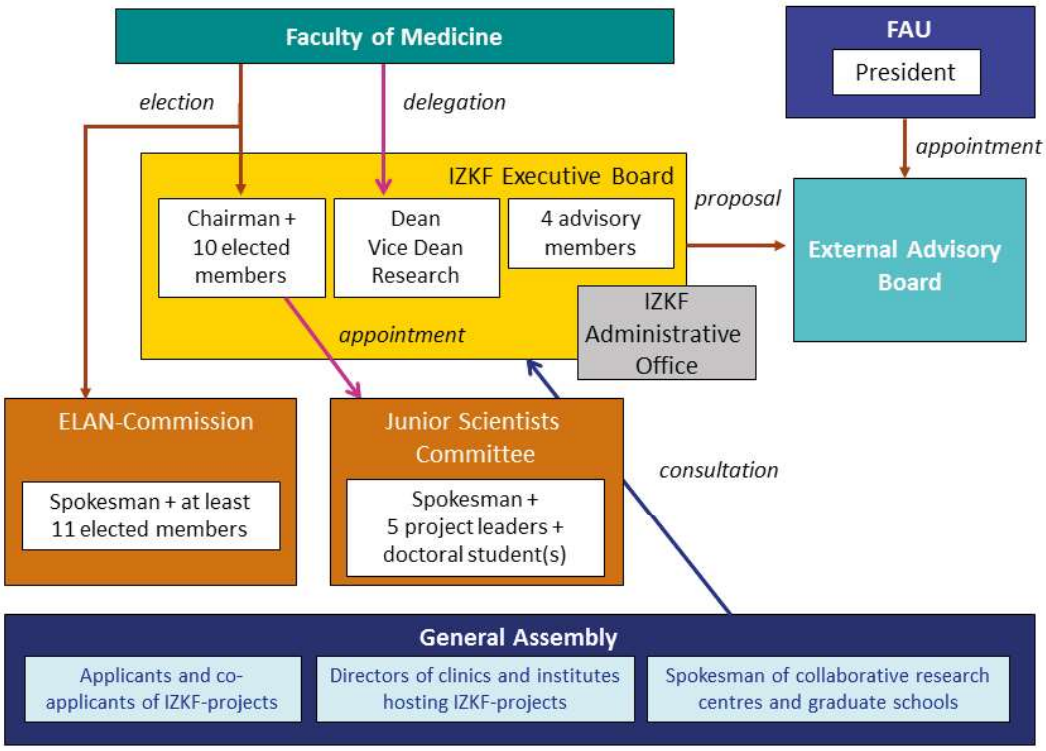
Cumulative Impact Factor **341.475**
Average Impact Factor per publication **7.941**
Average publications per project **0,9**
10 publication with an IF more than 10

About us

Structure of the IZKF

The IZKF is a self-organised structure within the Faculty of Medicine. The IZKF has a set of written rules and regulations approved by the Faculty of Medicine. All rules and regulations are continuously reviewed and revised, if necessary. The Statutes of the IZKF regulate the status, tasks and objectives of the IZKF as well as the competence and composition of the committees. The Rules of Procedure specify the application procedure, the funding and duration of the projects as well as the decision-making process between the Chairman, the Management Board and the External Advisory Board. Finally, the Advisory Board regulations regulate the IZKF's cooperation with the Advisory Board in detail. All regulations are available at the IZKF Homepage.

Governing bodies include the General Assembly, the Management Board, the Junior Scientists Committee, the ELAN-Commission and the External Scientific Advisory Board (SAB).



Governance of the IZKF.

Staff and bodies

The Management Board is the general steering commission of the IZKF. It is responsible for developing the scientific programme, controlling the financial framework and allocating resources to projects as well as ensuring that results are reported. It is composed of up to 13 members with voting rights, up to 11 elected by the Faculty of Medicine for a three year period and two ex-officio members from the Faculty of Medicine as well as four advisory members from the University Hospital and the University. Five annual meetings are held and decisions are taken by simple qualified majority. Elected members include the Chairman who is responsible for daily operations with the support of the Administrative Office. Re-election is possible for all members of the Board.

Programmes and the financial framework are reviewed and approved by the External Scientific Advisory Board. This body meets on site every two or three years to oversee the general development of the IZKF and the proposed projects.

The Board consists of at least 10 internationally recognized scientists (16: 31/12/2019) from universities and research institutes led by an elected chairperson, who is appointed for a period of 4 years with a maximum term of office of 8 years. The members of the External Scientific Advisory Board are appointed by the University President, upon the proposal of the Management Board for a period of six years. The maximum term of office in the External Scientific Advisory Board is usually 12, as an exception 13 years.

Since the merger of the IZKF and the ELAN Fund under the umbrella of the IZKF, the ELAN-Commission has been a part of the IZKF body. It is responsible for reviewing pilot projects and assists in the selection of advanced and junior projects. It consists of the spokesman for pilot projects (ELAN) and at least 11 further members all elected by the Faculty of Medicine for a period of three years. A re-election is possible. The chairperson of the ELAN-Commission is an elected member of the IZKF Board.

The Junior Scientists Committee supports the Management Board in establishing and supervising career development programmes for young scientists. It assigns the MD-Thesis scholarships and organises the IZKF Research Training Group. In addition, it participates in the internal review process for project funding and for laboratory rotations.

It is composed of the spokesman (who also belongs to the IZKF-Management Board) for promotion of young researchers elected by the Faculty of Medicine, five project leaders, three from advanced projects, one from junior projects and one of the junior research group leaders and at least one, but generally two representatives from the doctoral students, who will be chosen from among the Jour Fixe speakers. The members are appointed for a period of 3 years by the Board of the IZKF. The maximum term of office is 6 years.

About us

The most recent committee of the IZKF is the Clinician Scientist Programme Commission (CSP-Commission). This three-member commission, which is made up of research-active physicians, accompanies the Clinician Scientist Programme of the IZKF in terms of organisation and content and makes recommendations regarding the admission of new applicants to the Clinician Scientist Programme. The members of the CSP-Commission are appointed by the IZKF Management Board for a period of 3 years. One of the members of the commission shall be a member of the Management Board. The IZKF Management Board also appoints the Chairman of the CSP-Commission.

The IZKF-Administrative Office is responsible for the administrative and financial management of the IZKF. Its main focus lies on the assistance of the Chairman, the Management Board and the Junior Scientists Committee, the preparation and follow-up of IZKF Committee meetings, handling of IZKF application procedures, internal communication (ongoing, website, annual report, informative events for new project leaders), account management and financial controlling as well as the organisation of events. The IZKF-Administrative Office is an integral part of the Research Funding Department of the Finance Department of the University Hospital.

The General Assembly convenes once a year to discuss the annual report of the chairman and to contribute proposals for the further development of the IZKF. The members are all project leaders, the directors of clinics and institutes receiving funding, and the speakers of all local collaborative research centers and graduate schools. The speakers from the Jour fixe of the IZKF-RTG regularly participate in the general meetings.

In 2019 some changes in the various Committees were experienced. Prof. Reis resigned from his position as Chairman of the Management Board. Prof. Wegner is now the new Head, supported by four new colleagues: Prof. Bozec as the new Vice-Chair, Prof. Mougiakakos, Prof. Neurath and Prof. Schiffer. Prof. Schüttler has resigned as Dean of the Faculty of Medicine, his successor is Prof. Neurath.

We would like to thank Prof. Reis for filling the position of Chairman of the Management Board for his commitment and efforts in the past 12 years.

There also have been changes in the External Scientific Advisory Board. We would like to thank Prof. Sendtner for his advice and in his function as Chairman. Prof. Seufferlein was appointed as his follower and Prof. Kuhlmann as Vice-Chair.

Also the ELAN-Commission changed. Prof. Brabletz resigned and we would like to thank him for his efforts and support. In addition to Prof. Brabletz, also Prof. Cesnjevar, Prof. Fasching and Prof. Überla resigned from the ELAN-Commission. The ELAN-Commission is now headed by Prof. Reis, supported by four new colleagues: Prof. Engel, Prof. Fejtova, Prof. Hellerbrand and Prof. Waldner.

External Advisory Board (from left to right): Prof. Seufferlein, Prof. Siebert, Prof. Busch, Prof. Sendtner, Prof. Katschinski, Prof. Kalinke, Prof. Tiegs, Prof. Pavenstädt, Prof. Sorokin, Prof. Hengel, Prof. Rieß, Prof. Prinz

Organisation of the IZKF Research Training Group

All members regularly participate in the Jour Fixe (JF) once a month. Due to the broad thematic range of the doctoral theses at the IZKF, several Jour Fixes are held, which are at the moment

- Immunology, infection, kidney and circulatory research (Ink)
- Neurology (Neuro)
- Onkology (Onko) and
- DigIT

Each JF is supervised by one to two spokespersons from the rank of doctoral students, who are elected from among the participants for a period of 2-3 years as a rule. Usually, a new election takes place at the end of the doctoral thesis of the respective spokesperson. In addition to the spokespersons, each established JF has a scientific head from the rank of appointed professors.

Jour Fixe Ink (Immunology, Infection, Renal and Vascular Research; former T(h)ink)

Scientific Head

Prof. Becker, Department of Medicine 1

Spokespersons

Christina-Jasmin Bayerlein, Institute of Microbiology

Katrin Peckert, Department of Immune Modulation

At the Jour Fixe INK, doctoral fellows working in the areas of immunology, infection, renal and vascular research will present the progress and results of their respective doctoral projects. The seminar is held in English and takes place once a month. It promotes both the transfer of knowledge between doctoral fellows in the different fields and the presentation and discussion skills in front of an audience.

Jour Fixe Neuro

Scientific Head

Prof. Dieter Chichung Lie, Institute of Biochemistry

Spokespersons

Wolfrat Bachert, Institute of Biochemistry

Judith Stemick, Department of Molecular Neurology

The neuroscientific doctoral fellows of the FAU Erlangen-Nuremberg meet monthly for the Jour Fixe „Neuroscience“, at which the doctoral fellows discuss new methods and technologies in addition to their respective doctoral projects. The programme of the Jour Fixe is solely organised by the doctoral students.

Jour Fixe Onko

Scientific Head

Prof. Anja Bosserhoff, Institute of Biochemistry

Spokespersons

Tatjana Seitz, Institute of Biochemistry

Kerstin Hübner, Institute of Pathology

In the Oncology Jour Fixe, doctoral fellows focusing on research in different fields of oncology discuss ongoing work as well as new approaches. Every participant presents her/his own project once a year in the form of a progress report. The topics of this seminar range from basic research in various cancer entities to clinical studies and targeted therapies.

Jour Fixe DigIT

Scientific Head

Prof. Olaf Gefeller, Institute of Medical Informatics, Biometry and Epidemiology

Spokespersons

Colin Griesbach, Institute of Medical Informatics, Biometry and Epidemiology

Anja Rappl, Institute of Medical Informatics, Biometry and Epidemiology

The JF DigIT is aimed at doctoral students with a data-analytical methodical approach. All participating institutions assign their self-conception to life sciences on the basis of their research orientation, even if in some doctoral projects there are clear references to other fields of science such as mathematics/statistics, computer science, physics and electrical engineering.

About us

Committees

Management Board

Chairman

Prof. Dr. André Reis, Institute of Human Genetics (till 09/2019)
Prof. Dr. Michael Wegner, Institute of Biochemistry (since 10/2019)

Deputy Chairman

Prof. Dr. Michael Wegner, Institute of Biochemistry (till 09/2019)
Prof. Dr. Aline Bozec, Department of Medicine 3 (since 10/2019)

Members

Prof. Dr. Christoph Becker, Department of Medicine 1
Prof. Dr. Christian Bogdan, Institute of Clinical Microbiology, Immunology and Hygiene
Prof. Dr. Anja Bosserhoff, Institute of Biochemistry
Prof. Dr. Thomas Brabletz, Chair of Experimental Medicine I (till 09/2019)
Prof. Dr. Johann Helmut Brandstätter, Division of Animal Physiology
Prof. Dr. Dr. Raymund Horch, Department of Plastic and Hand Surgery
Prof. Dr. Andreas Mackensen, Department of Medicine 5 (till 09/2019)
Prof. Dr. Dimitrios Mougiakakos, Department of Medicine 5 (since 10/2019)
Prof. Dr. Markus Neurath, Department of Medicine 5 (since 10/2019)
Prof. Dr. André Reis, Institute of Human Genetics (since 10/2019)
Prof. Dr. Mario Schiffer, Department of Medicine 4 (since 10/2019)
Prof. Dr. Jürgen Winkler, Department of Molecular Neurology

Consultative Members

Prof. Dr. Joachim Hornegger, President of the FAU
Christian Zens, Head of Administration of the FAU
Prof. Dr. Dr. Heinrich Iro, Medical Director of the University Hospital Erlangen
Dr. Albrecht Bender, Head of Administration of the University Hospital Erlangen

Prof. Dr. Wegner

Prof. Dr. Bozec

Prof. Dr. Becker

Prof. Dr. Bogdan

Prof. Dr. Bosserhoff

Prof. Dr. Brandstätter

Prof. Dr. Dr. Horch

Prof. Dr. Mougiakakos

Prof. Dr. Dr. Neurath

Prof. Dr. Reis

Prof. Dr. Schiffer

Prof. Dr. Winkler

Prof. Dr. Hornegger

Zens

Prof. Dr. Dr. Iro

Dr. Bender

Current members of the Management Board

About us

External Scientific Advisory Board

Chairman

Prof. Dr. Michael Sendtner,
University Hospital Würzburg - Institute for Clinical Neurobiology (till 11/2019)

Prof. Dr. Thomas Seufferlein,
University Hospital Ulm - Internal Medicine I (since 11/2019)

Deputy Chairman

Prof. Dr. Tanja Kuhlmann,
University Hospital Münster, Institute of Neuropathology (since 11/2019)

Members

Prof. Dr. Dirk Busch,
Technical University of Munich, Institute for Medical Microbiology, Immunology and Hygiene

Prof. Dr. Hartmut Hengel,
Freiburg University Hospital - Department of Virology

Prof. Dr. Ulrich Kalinke,
TWINCORE, Centre for Experimental and Clinical Infection Research

Prof. Dr. Thomas Kamradt,
Jena University Hospital, Institute of Immunology

Prof. Dr. Dörthe Katschinski,
Göttingen University Medical Center - Department of Cardiovascular Physiology

Prof. Dr. Holger Moch,
University Hospital Zurich, Institute of Pathology and Molecular Pathology

Prof. Dr. Hermann Pavenstädt,
Münster University Hospital - Internal Medicine, Department of Nephrology and Rheumatology

Prof. Dr. Jörg Prinz,
LMU München, Department of Dermatology and Allergology

Prof. Dr. Olaf Rieß,
University of Tübingen - Institute of Human Genetics

Prof. Dr. Jörg B. Schulz,
University Hospital Aachen - Department of Neurology

Prof. Dr. Michael Sendtner,
University Hospital Würzburg - Institute for Clinical Neurobiology (till 12/2019)

Prof. Dr. Reiner Siebert,
University Hospital Ulm, Institute of Human Genetics

Prof. Dr. Lydia Sorokin,
University of Münster, Institute of Physiological Chemistry and Pathobiochemistry

Prof. Dr. Gisa Tiegs,
Hamburg-Eppendorf University Medical Center - Institute of Experimental Immunology and Hepatology

Prof. Dr. Seufferlein

Prof. Dr. Kuhlmann

Prof. Dr. Busch

Prof. Dr. Hengel

Prof. Dr. Kalinke

Prof. Dr. Kamradt

Prof. Dr. Katschinski

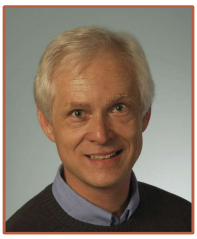
Prof. Dr. Moch

Prof. Dr. Pavenstädt

Prof. Dr. Prinz

Prof. Dr. Rieß

Prof. Dr. Schulz



Prof. Dr. Sendtner

Prof. Dr. Siebert

Prof. Dr. Sorokin

Prof. Dr. Tiegs

External Scientific Advisory Board
(as of 31st December 2019)

About us

ELAN-Commission

Spokesman for pilot projects (ELAN)

Prof. Dr. Thomas Brabletz, Chair of Experimental Medicine I (till 09/2019)
Prof. Dr. André Reis, Institute of Human Genetics (since 10/2019)

Members

Prof. Dr. Tobias Bäuerle, Institute of Radiology
Prof. Dr. Jürgen Behrens, Chair of Experimental Medicine II
Prof. Dr. Robert Cesnjevar, Department pf Paediatric Cardiac Surgery (till 09/2019)
Prof. Dr. Felix Engel, Department of Nephropathology
Prof. Dr. Yesim Erim, Department of Psychosomatic Medicine and Psychotherapy
Prof. Dr. Peter Fasching, Department of Obstetrics and Gynaecology (till 10/2019)
Prof. Dr. Anna Fejtova, Department Psychiatry and Psychotherapy (since 10/2019)
Prof. Dr. Martin Fromm, Chair of Clinical Pharmacology and Clinical Toxicology
Prof. Dr. Claus Hellerbrand, Institute of Biochemistry (since 10/2019)
Prof. Dr. Gerhard Krönke, Department of Medicine 3
Prof. Dr. Christian Pilarsky, Department of Surgery
Prof. Dr. Alexander Steinkasserer, Department of Immune Modulation
Prof. Dr. Regina, Trollmann, Department of Paediatrics and Adolescent Medicine (till 09/2019)
Prof. Dr. Klaus Überla, Institute of Clinical and Molecular Virology (till 09/2019)
Prof. Dr. Maximilian Waldner, Department of Medicine 1 (since 10/2019)
Prof. Dr. Beate Winner, Department of Stem Cell Biology

Prof. Dr. Reis

Prof. Dr. Bäuerle

Prof. Dr. Behrens

Prof. Dr. Engel

Prof. Dr. Erim

Prof. Dr. Fejtova

Prof. Dr. Fromm

Prof. Dr. Hellerbrand

Prof. Dr. Krönke

Prof. Dr. Pilarsky

Prof. Dr. Steinkasserer

Prof. Dr. Waldner

Prof. Dr. Winner

Current members of the ELAN Commission

About us

Junior Scientists Committee

Prof. Dr. Becker

Dr. Dulin

Prof. Dr. Engel

Griesbach

PD Dr. Müller-Deile

Dr. Nganou Makamdop

Seitz

Prof. Dr. Zimmermann

Current members of the Junior Scientists Committee

Spokesman for career development programmes

Prof. Dr. Christoph Becker, Department of Medicine 1

Members

Prof. Dr. Aline Bozec, Department of Medicine 3 (till 09/2019)

Prof. Dr. Paolo Ceppi, IZKF Junior Research Group 1 (till 09/2019)

Dr. David Dulin, IZKF Junior Research Group 2 (since 10/2019)

Prof. Dr. Felix Engel, Department of Nephropathology

Colin Griesbach, Department of Medical Informatics, Biometry and Epidemiology (since 12/19)

PD Dr. Janina Müller-Deile, Department of Medicine 4 (since 12/19)

Dr. Christiane Krystelle Nganou Makamdop, Institute of Clinical and Molecular Virology

Tatjana Seitz, Institute of Biochemistry (since 04/2019)

Iris Stolzer, Department of Medicine 1 (till 03/2019)

Prof. Dr. Katharina Zimmermann, Department of Anaesthesiology

Clinician Scientist Programme Commission


Prof. Dr. Mougiakakos


Prof. Dr. Dr. Zweier


Prof. Dr. Winkler

Current members of the CSP-Commission

Spokesman for Clinician Scientist Programme

Prof. Dr. Dr. Christiane Zweier, Institute of Human Genetics

Members

Prof. Dr. Dimitrios Mougiakakos, Department of Medicine 5

Prof. Dr. Jürgen Winkler, Department of Molecular Neurology

Administrative Office


Meyerhöfer-Klee


Dr. Faber


Neufang


Reichel

Current staff of the Administrative Office

Manager

Dr. Katrin Faber

IZKF Administration

Anne Reichel

Kathrin Neufang

Bianca Meyerhöfer-Klee

About us

General Assembly

Surname	Name
Amann	Kerstin
Bäuerle	Tobias
Bachert	Wolfrat
Balk	Matthias
Bayerlein	Jasmin
Becker	Christoph
Beckmann	Matthias W.
Behrens	Jürgen
Bender	Albrecht
Berking	Carola
Bernkopf	Dominic
Blümcke	Ingmar
Bogdan	Christian
Boßerhoff	Anja
Bozec	Aline
Brabletz	Thomas
Brandstätter	Johann Helmut
Britzen-Laurent	Nathalie
Büttner-Herold	Maike
Buchfelder	Michael
Ceppi	Paolo
Chen	Chih-Wei
Chiriac	Mircea
Dietrich	Peter
Distler	Jörg
Dudziak	Diana
Dulin	David
Eberhardt	Esther
Engel	Felix
Ensser	Armin
Erim	Yesim
Fejtova	Anna
Finotto	Susetta
Frohwitter	Gesche
Fromm	Martin
Full	Florian

Surname	Name
Gefeller	Olaf
Gölz	Lina
Full	Florian
Gefeller	Olaf
Gölz	Lina
Gramberg	Thomas
Grampp	Steffen
Gregor	Anne
Griesbach	Colin
Grosche	Linda
Grützmann	Robert
Günther	Claudia
Hartmann	Arndt
Heckel	Maria
Hellerbrand	Claus
Hessenauer	Maximilian
Hildner	Kai
Horch	Raymund
Hornegger	Joachim
Hübner	Kerstin
Hüffmeier	Ulrike
Iro	Heinrich
Jabari	Samir
Jitschin	Regina
Jobst-Schwan	Tilman
Kachler	Katerina
Kannenkeril	Dennis
Kengelbach-Weigand	Annika
Kesting	Marco
Klingberg	Anika
Korbmacher	Christoph
Kornhuber	Johannes
Krach	Florian
Kraus	Andre
Kretschmann	Sascha
Krönke	Gerhard

Surname	Name
Kuwert	Torsten
Lehmann	Christian
Leone	Marina
Lie	Dieter Chichung
Mackensen	Andreas
Mahapatro	Mousumi
Marschall	Manfred
Maslarova	Anna
Meng	Xianyi
Metzler	Markus
Meyer	Sebastian
Mougiakakos	Dimitrios
Müller-Deile	Janina
Müller	Fabian
Nganou Makamdop	Christiane Krystelle
Nagel	Armin
Neurath	Markus
Ostgathe	Christoph
Patankar	Jay
Peckert	Katrin
Pfeifle	Rene
Pilarsky	Christian
Raimondo	Maria Gabriella
Ramming	Andreas
Rappl	Anja
Regensburger	Adrian
Reis	André
Rottensteiner	Ulrike
Ruder	Barbara
Salar	Seda
Schäffner	Iris
Schett	Georg
Schiffer	Mario
Schleicher	Ulrike
Schmidkonz	Christian
Schödel	Johannes
Schuhwerk	Harald
Schüttler	Jürgen
Schwab	Stefan
Schwartz	Christian
Seidel	Thomas
Seitz	Tatjana
Sembill	Jochen
Sikic	Danijel
Simon	David
Spörl	Silvia
Steinkasserer	Alexander
Stemick	Judith
Sticht	Heinrich
Stolzer	Iris
Strick	Reiner
Stürzl	Michael
Tenbusch	Matthias
Tenkerian	Clara
Thoma-Kreß	Andrea
Überla	Klaus
Uder	Michael
Waldmann	Elisabeth
Waldner	Maximilian
Wegner	Michael
Winkler	Jürgen
Winner	Beate
Wölfle	Joachim
Wullich	Bernd
Zaiss	Mario
Zens	Christian
Zimmermann	Katharina
Zweier	Christiane
Zundler	Sebastian

General Assembly of the IZKF (as of 10th December 2019)

About us

Financial Report 2019

Since 2004, the IZKF has been fully supported by intramural funds. The main financial contribution is given by the Faculty of Medicine. Additional contributions are received from the FAU.

Part of the expenditures of 2019 were financed from residual funds of the previous years.

Revenues	
Support of the Medical Faculty	5,469 K€
Support of the University	364 K€
Other revenues	57 k€
Total revenues 2019	**5,890 K€**
Expenditures	
Advanced projects	1,053 K€
Pilot projects	750 K€
Career development	2,242 K€
thereof junior research groups	644 K€
thereof junior projects	1,022 K€
thereof laboratory rotations	390 K€
thereof MD-thesis scholarships	160 K€
thereof research training groups	26 K€
Central projects	111 K€
Administration	218 K€
Total expenditures 2019	**4,374 K€**

Revenues and expenditures 2019

Output and Evaluation

Various parameters are used to assess compliance with the mission of the IZKF in advancing clinically oriented research at the Faculty. Scientific publications and academic success of young scientists are the most obvious and straightforward ones. Additionally, the acquisition of extramural funding is an explicit objective of the IZKF. Furthermore, patents, scientific prizes and offers of professorships are relevant parameters. Other important parameters for the IZKF are the number of different institutions and scientists, who are involved in the IZKF, the number of interdisciplinary projects as well as the number of joint publications.

In the reporting period 49 scientific projects were actively running: 24 advanced projects, 23 junior projects and 2 junior research groups. In addition, 7 junior projects started their work in 2019 (4) or in the beginning of 2020 (3). These 49 funded scientific projects published 43 original articles in 2019 resulting in an average of 0.9 publications per project. The cumulative impact factor (IF) was 341.475, averaging 7.941 per publication. The high quality of many of these publications is reflected in 10 publications with an IF of more than 10. Being part of the IZKF allows intensive networking and direct access to collaborations, which can be seen in 5 publications that were generated in a cooperation of multiple projects. Additional articles of finalised projects are in preparation, submitted or accepted. Publications that have already been accepted are listed in the corresponding final reports.

Intense academic activity within the IZKF advanced and junior projects is reflected in 10 master theses, 53 doctoral theses and 4 habilitations that were in progress or finalised in 2019. A total of 60 project leaders and 55 employed scientists are involved in 47 scientific projects (advanced and junior) funded by the IZKF.

Some IZKF project leaders were able to achieve outstanding results. 11 prizes were awarded to IZKF project leaders. Three professorships were offered.

In many instances funding by the IZKF starts at an early phase of the project, thus it must be considered as a high risk funding programme. It is nevertheless reassuring that most of the projects are successful and many of them are continued after the termination of intramural funding. To support this with figures, results of a detailed survey of acquired third-party funding by the IZKF-projects are given on the next pages.

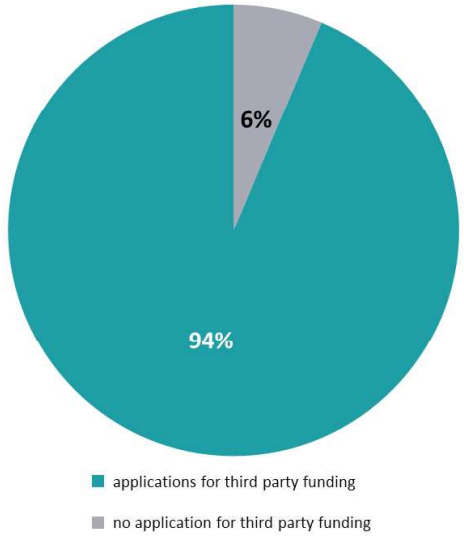
Beginning with the funding period of 2010-2013, grants were awarded for a period of 30 months with an extension by 6 months, if these projects are submitted for external funding. Within the funding period of 2013-2016 all projects submitted third party funds applications and therefore received the 6 months funding extension. Of the 31 projects from the 2016-2019 funding period, 29 (94%) have applied for project extensions.

When considering the last three funding periods (2010-2019), 78 projects were funded by the IZKF of which 73 (94%) submitted third party funds applica-

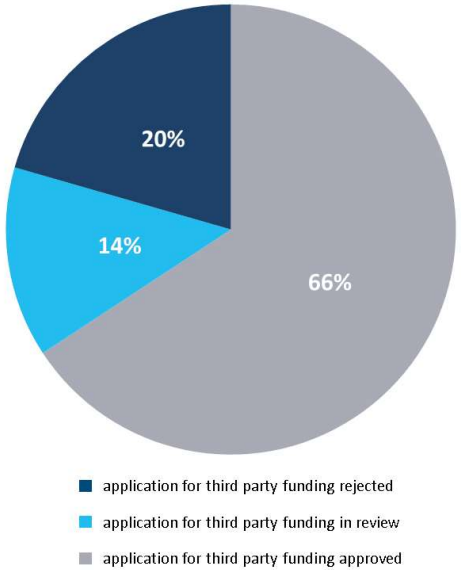
tions. 48 of these projects (66%) were granted extramural funding, only 15 (20%) were not funded and 10 (14%) are still in review.

From the funding period 2013-2016, which was completed last year, 30 (97%) projects have submitted an application to an external funding institution.

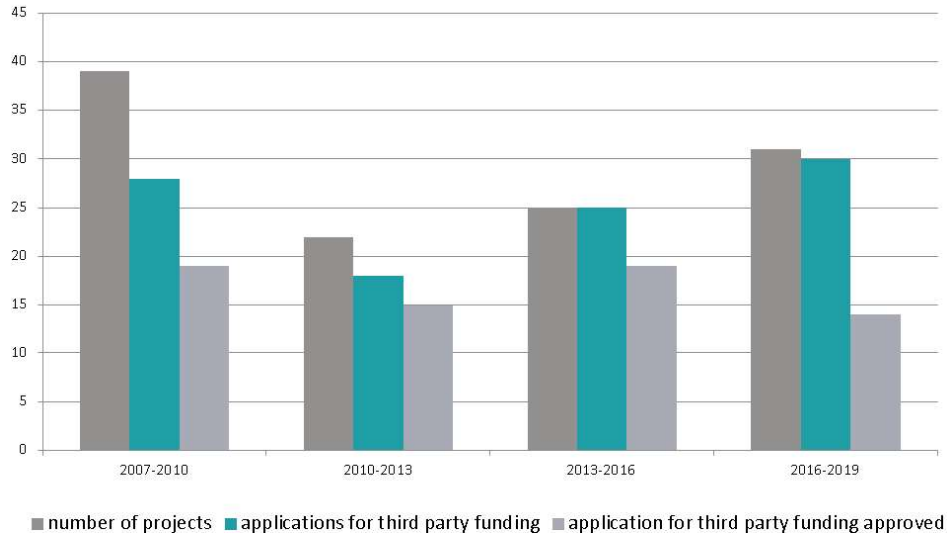
Similarly, the junior projects lead to a high number of extramural funding applications with a very high success rate. This development has been stable over the entire duration of the programme.



Applications for third-party funding submitted by advanced projects between 2010 and 2019.

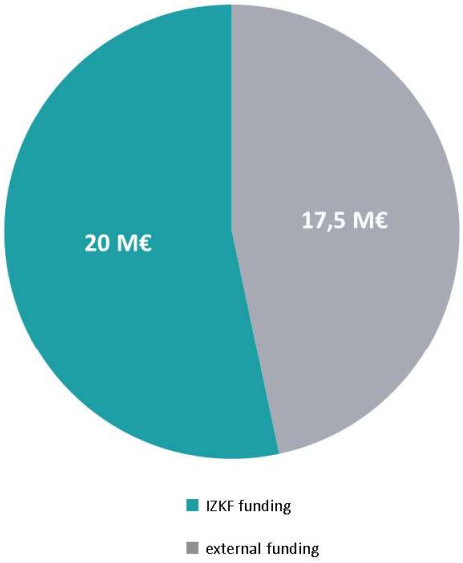


Approved applications for third-party funding of advanced projects between 2010 and 2019.

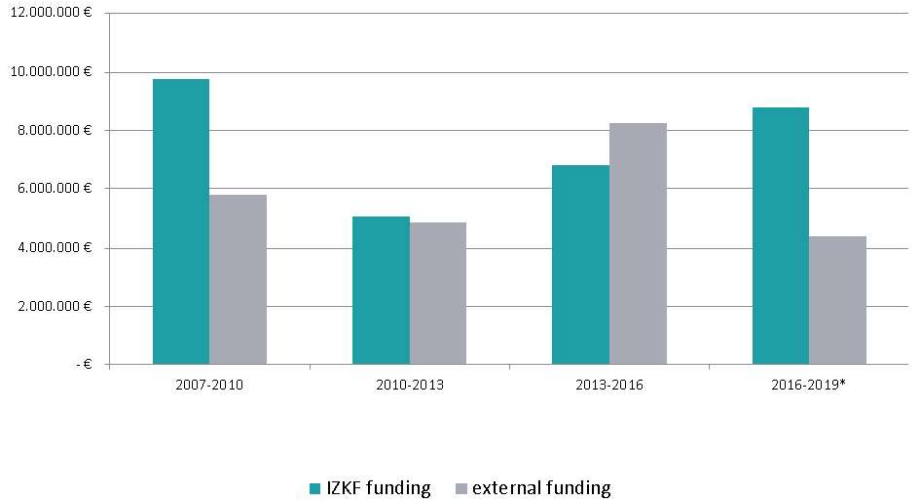


This column graph compares the number of advanced projects with the number for the submitted and approved applications for external funding in each funding period.

About us

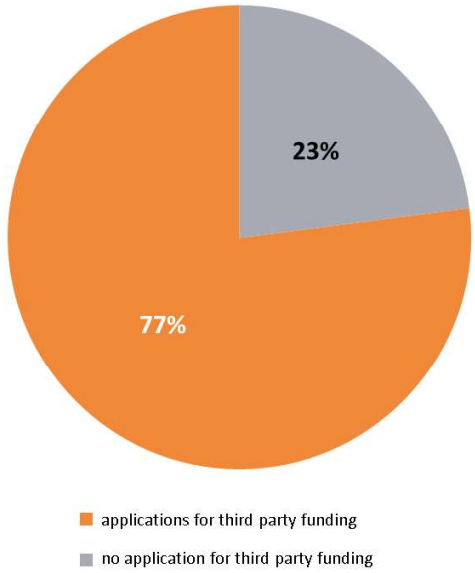


External funding received from advanced projects between 2010 and 2019.

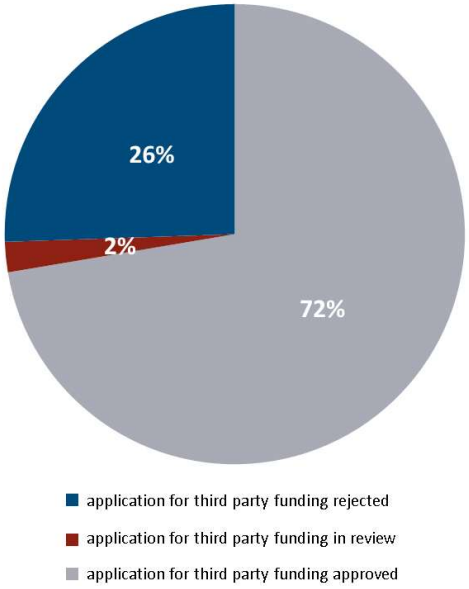


External funding received from advanced projects between 2007 and 2019.

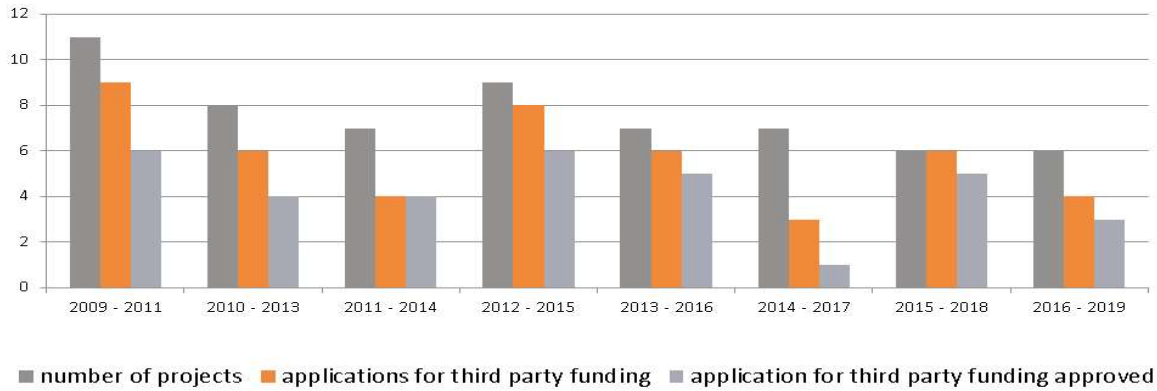
Acquisition of third-party funding by junior projects



Applications for third-party funding submitted by junior projects (projects initiated between 2009 and 2016).

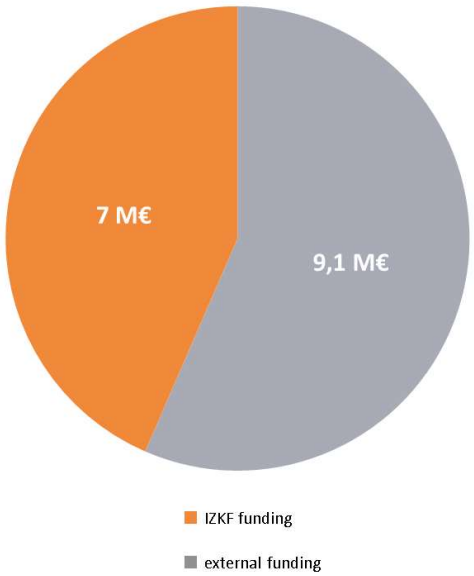


Approved applications for third-party funding of junior projects (projects initiated between 2009 and 2016).

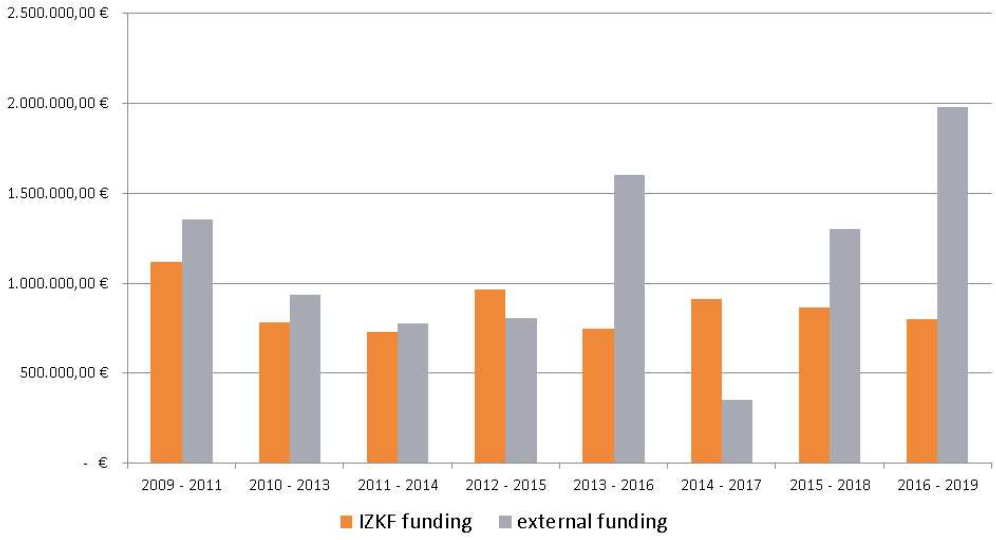


Success-rate of junior projects. Initiated 2009-2016.

About us

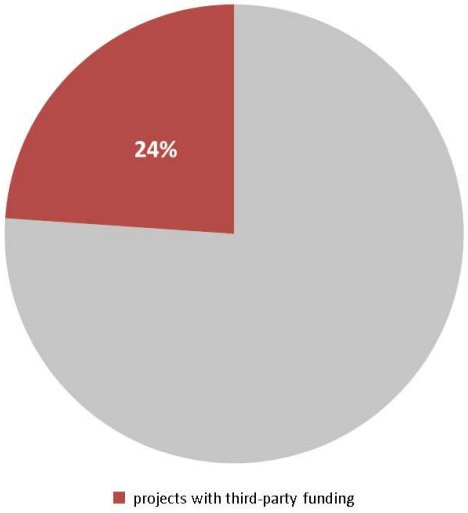


External funding received from junior projects between 2010 and 2019.

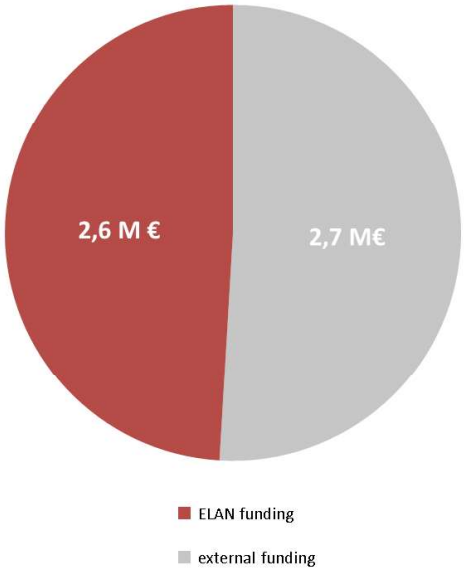


External funding received from junior projects between 2007 and 2019.

Acquisition of third-party funding by pilot projects

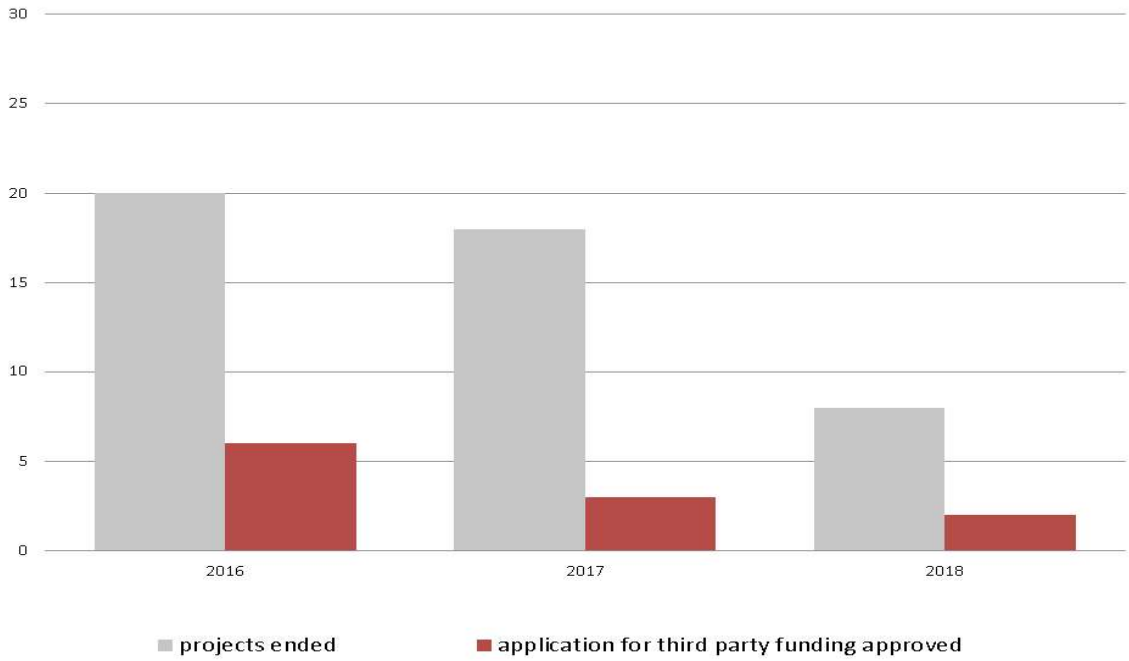


Pilot projects with third-party funding (projects completed between 2016 and 2018).

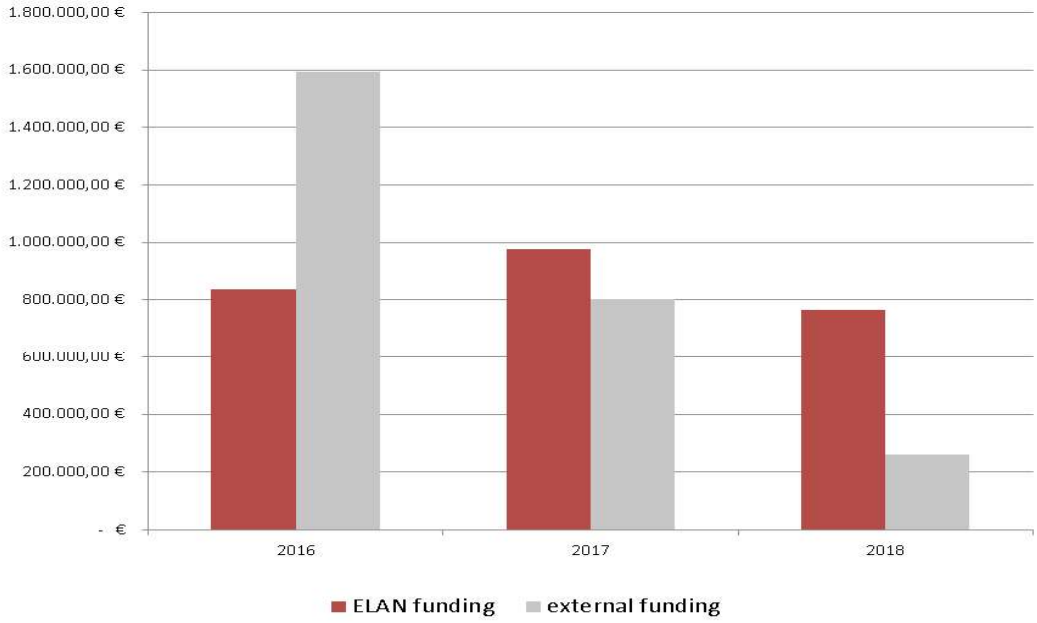


External funding received from all pilot projects completed between 2016 and 2018.

About us



Success-rate of pilot projects. Further applications of projects, initiated in 2018, are planned.



External funding from pilot projects completed between 2016 and 2018.

Scientific Reports Advanced Projects

Scientific Reports Junior Groups/Projects

Scientific Reports Pilot Projects

A63 - Final Report

01/07/2016 - 30/06/2019

Mechanisms of TNF-Mediated Control of Intracellular Pathogens in Mice and Man

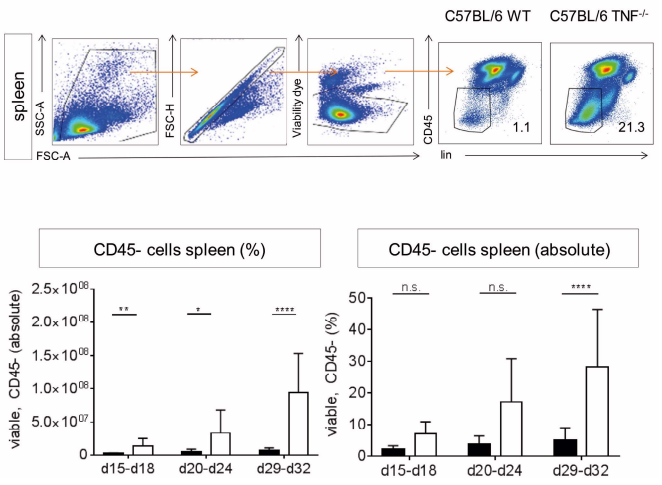
Prof. Dr. Christian Bogdan, Institute of Clinical Microbiology, Immunology and Hygiene

Neutralization or deletion of tumor necrosis factor (TNF) causes loss of control of intracellular pathogens in mice and humans, but the underlying mechanisms are incompletely understood. This project aims to define mechanisms by which TNF protects from progressive cutaneous leishmaniasis. In parallel, TNF-regulated protective versus disease-mediating pathways will be evaluated in immune cells of patients with rheumatoid arthritis (RA) before and after treatment with TNF-antagonists.

Tumor necrosis factor (TNF)-deficient C57BL/6 mice fail to control a local cutaneous infection with the intracellular pathogen *Leishmania (L.)* major and develop progressive and fatal visceral disease, despite a fully preserved Th1 immune response and unaltered expression of type 2 nitric oxide (NO) synthase (NOS2). NOS2 metabolizes L-arginine to citrulline and NO, which exerts antimicrobial and immunoregulatory effects. The activity of NOS2 is antagonized by arginase (Arg1) that converts the same substrate L-arginine into urea and ornithine.

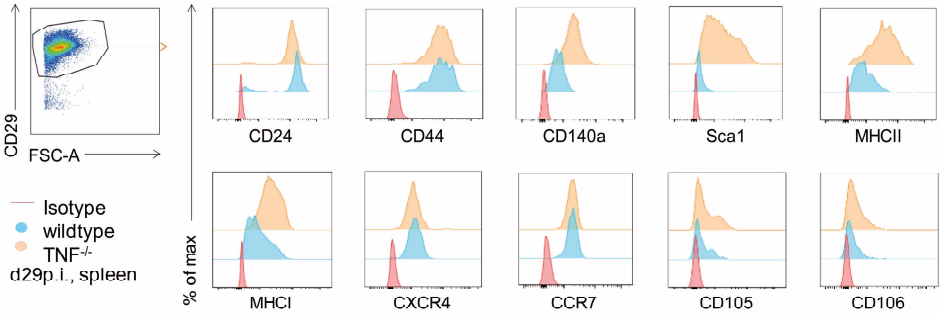
We previously observed that the lack of TNF resulted in hyperexpression of Arg1, which led to an impaired production of NO at the sites of infection. To address further the functional role of Arg1 in $TNF^{-/-}$ mice, we generated TNF- and Arg1-double-deficient C57BL/6 mice. Surprisingly, $TNF^{-/-}Tie2Cre^{+/-}Arg1^{fl/fl}$ mice still developed progressive cutaneous leishmaniasis comparable to $TNF^{-/-}$ mice, although the production of NO was restored in the infected tissues. This indicated that mechanisms other than Arg1 upregulation contribute to the non-healing course in L. major-infected $TNF^{-/-}$ mice.

As *L. major*-infected $TNF^{-/-}$ mice succumbed to visceral disease with high splenic parasite loads, we performed RNASeq analysis of spleens from *L. major*-infected $TNF^{-/-}$ vs. WT mice at two different time points p.i. to discover "novel" TNF-regulated antimicrobial effectors. Based on these data we detected a significant expansion of a non-hematopoietic, CD45- cell population in the spleen of infected $TNF^{-/-}$ mice. Flow cytometry analyses revealed that these CD45- cells neither represented endothelial cells nor fibroblastic reticular cells or hematopoietic stem cells. Instead, the marker profile of this cell population (positive: CD29, CD44, CD24, CD140a, CXCR4, CCR7, Sca1, MHCII, MHCI; negative: e.g. CD3, CD19, CD11b, ckit) was reminiscent of mesenchymal stem cells (MSCs). The MSC-like cells were also present in spleens of L. major-infected WT mice, but to a much lower extent. Additionally, the expression of Sca1, MHC class I and II on MSCs was stronger in mice lacking TNF as compared to WT mice, indicating an increased activation status of the $TNF^{-/-}$ MSCs. The MSC-like cells do not seem to function as host cells of Leishmania. To address a potential immunomodulatory role, RNASeq analysis of sorted MSCs was performed the results of which are currently evaluated.



Flow cytometric analysis of CD45-negative cells in spleens of L. major infected TNF-/- vs WT control mice. Gating strategy, percentage and absolute cell numbers of the CD45-negative cells during infection are given.

Prof. Dr. Bogdan



Surface marker profile of CD45- lineage- cells in the spleen of L. major infected TNF$^{-/-}$ mice.

We also continue to investigate the regulation of Arg1 by TNF in humans. So far, we have been unable to detect definitive changes in the expression Arg1 in peripheral blood mononuclear cells and neutrophils from patients with rheumatoid arthritis before and during treatment with TNF-antagonists. In an unbiased approach, RNASeq analysis of whole blood samples of these patients was performed to detect genes regulated by the blockade of TNF. Due to very high heterogeneity and so far low number of patients we could not yet identify common TNF-dependent pathways.

Contact:
Prof. Dr. Bogdan
phone: +49 9131 85 22551
e-mail: christian.bogdan@uk-erlangen.de

Invited lectures

Bogdan C. Cutaneous leishmaniasis: new aspects of pathogenesis and disease (plenary lecture). Conference on Tropical Medicine and Global Health. Ludwig-Maximilians-Universität München, April 4-6, 2019

Bogdan C. Arginine 1, nitric oxide synthase 2 and TNF: three players in cutaneous leishmaniasis (plenary lecture). Symposium on Oxidative Stress and Implications for Inflammation and Immune Response, Kopenhagen, May 14-15, 2019

Publications during funding period

Molkara S, Poursoltani E, Stahl KW, Maleki M, Khamesipour A, Bogdan C, Salehi M, Goyonlo VM (2019) Salvage therapy with Sodium chlorosum (formerly DAC N-055) for cases of refractory lupoid cutaneous leishmaniasis: results from a compassionate use study with 0.09% Sodium chlorosum in amphiphilic basic cream. BMC Infect Dis. 19(1):1005. doi: 10.1186/s12879-019-4518-x

Hannemann N, Cao S, Eriksson D, Schnelzer A, Jordan J, Eberhardt M, Schleicher U, Rech J, Ramming A, Uebe S, Ekici A, Cañete JD, Chen X, Bäuerle T, Vera J, Bogdan C, Schett G, Bozec A (2019) Transcription factor Fra-1 targets arginase-1 to enhance macrophage-mediated inflammation in arthritis. J Clin Invest. 129(7):2669-2684. doi: 10.1172/JCI96832

Paduch K, Debus A, Rai B, Schleicher U, Bogdan C. (2019). Resolution of cutaneous leishmaniaisis and persistence of Leishmania major in the absence of arginase 1. J. Immunol.202(5):1453-1464

Bogdan C, Debus A, Sebald H, Rai B, Schäfer J, Obermeyer S, Schleicher U (2019) Experimental cutaneous leishmaniasis: mouse models for resolution of inflammation versus chronicity of disease. Methods Mol Biol. 1971:315-349. doi: 10.1007/978-1-4939-9210-2_18

Schleicher, U., Liese, J., Justies, N., Mischke, T., Haeberlein, S., Sebald, H., Kalinke, U., Weiss, S., and Bogdan, C (2018) Type I interferon signaling Is required for CpG-oligodesoxynucleotide-induced control of Leishmania major, but not for spontaneous cure of subcutaneous primary or secondary L. major Infection. Frontiers in Immunology 9: 79

Soulat D, Bogdan C (2017) Function of macrophage and parasite phosphatases in leishmaniasis. Frontiers in Immunology 8: 1-21 (epublished Dec 22) doi: 10.3389/fimmu.2017.01838

Leitherer S, Clos J, Liebler-Tenorio EM, Schleicher U, Bogdan C, Soulat D (2017) Characterization of the protein tyrosine phosphatase LmPRL-1 secreted by Leishmania major via the exosome pathway. Infection and Immunity 85(8): 1-19 (epublished July 19, 2017) doi: 10.1128/IAI.00084-17

Schleicher U, Paduch K, Debus A, Obermeyer S, König T, Kling JC, Ribechini E, Dudziak D, Mougiakakos D, Murray PJ, Ostuni R, Körner H, Bogdan C (2016) TNF-meditated restriction of arginase 1 expression in myeloid cells triggers type 2 NO synthase activity at the site of infection. Cell Reports 15(5): 1062-1075

A64 - Final Report

01/02/2016 - 31/01/2019

The tyrosine-protein phosphatase SHP2 regulates TGFβ-dependent activation of JAK2/STAT3 in fibrotic diseases

Prof. Dr. Jörg Distler, Prof. Dr. Georg Schett, Department of Medicine 3 – Rheumatology and Immunology

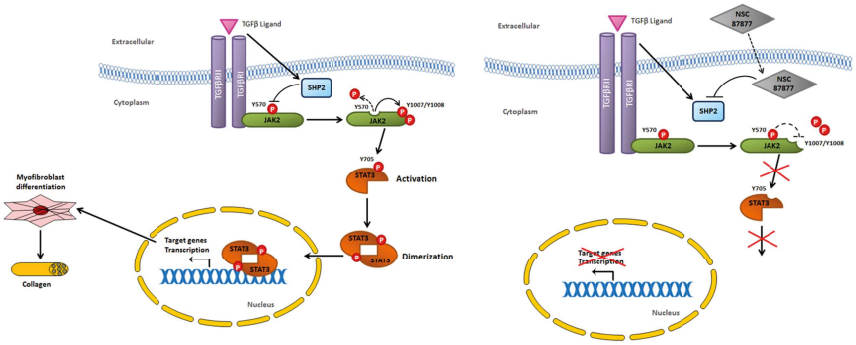
SHP2 is a ubiquitously expressed non-receptor tyrosine phosphatase with important regulatory effects on receptor tyrosine kinase-, cytokine- and G-protein coupled receptor signaling. Altered SHP2 activity due to mutations of the PTPN11 gene have been found in Noonan syndrome, juvenile myelomonocytic leukemia, and several types of human malignancies. We provide first evidence that SHP2 might also play a central role in the pathogenesis of fibrotic diseases such as Systemic Sclerosis (SSc) by inhibiting TGF-ß mediated activation of JAK2 / STAT3 signaling.

Uncontrolled activation of TGFβ signaling is a common denominator of fibrotic tissue remodeling. Here we characterize the tyrosine phosphatase SHP2 as a molecular checkpoint for TGFβ-induced JAK2/STAT3 signaling and as a potential target for the treatment of fibrosis. TGFβ stimulates the phosphatase activity of SHP2, although this effect is in part counterbalanced by inhibitory effects on SHP2 expression. Stimulation with TGFβ promotes recruitment of SHP2 to JAK2 in fibroblasts with subsequent dephosphorylation of JAK2 at Y570 and activation of STAT3. The effects of SHP2 on STAT3 activation translate into major regulatory effects of SHP2 on fibroblast activation and tissue fibrosis. Genetic or pharmacologic inactivation of SHP2 promotes accumulation of JAK2 phosphorylated at Y570, reduces JAK2/STAT3 signaling, inhibits TGFβ-induced fibroblast activation and ameliorates dermal and pulmonary fibrosis. In summary, we characterize SHP2 as a positive regulator of TGFβ-dependent activation of JAK2/STAT3 signaling. Genetic or pharmacologic inactivation of SHP2 inhibits JAK2/ STAT3 signaling, reduces fibroblast activation and ameliorates experimental fibrosis in several complementary models. Given the availability of potent SHP2 inhibitors, SHP2 might be a potential novel target for the treatment of fibrosis.

Contact:
Prof. Dr. Distler
phone: +49 9131 85 43008
e-mail: joerg.distler@uk-erlangen.de

Prof. Dr. Distler

Prof. Dr. Schett



Schematic summary of the role of SHP2 in TGFβ-induced fibroblast activation and tissue fibrosis.

Publications during funding period

Zehender A, Huang J, Györfi AH, Matei AE, Trinh-Minh T, Xu X, Li YN, Chen CW, Dees C, Beyer C, Gelse K, Zhang ZY, Bergmann C, Ramming A, Birchmeier W, Distler O, Schett G, Distler JHW (2018) The tyrosine phosphatase SHP2 controls TGFβ-induced STAT3 signaling to regulate fibroblast activation and fibrosis. Nat Commun. 14;9: 3259

Zhang Y, Pötter S, Chen CW, Liang R, Gelse K, Ludolph I, Horch RE, Distler O, Schett G, Distler JHW*, Dees C* (2018) Poly(ADP-ribose) polymerase-1 regulates fibroblast activation in systemic sclerosis. Ann Rheum Dis. 77: 744-751 * contributed equally

Soare A, Weber S, Maul L, Rauber S, Gheorghiu AM, Luber M, Houssni I, Kleyer A, von Pickardt G, Gado M, Simon D, Rech J, Schett G, Distler JHW, Ramming A (2018) Cutting Edge: Homeostasis of Innate Lymphoid Cells Is Imbalanced in Psoriatic Arthritis. J Immunol.15;200: 1249-1254

Matei AE, Beyer C, Györfi AH, Soare A, Chen CW, Dees C, Bergmann C, Ramming A, Friebe A, Hofmann F, Distler O, Schett G, Distler JHW (2018) Protein kinases G are essential downstream mediators of the antifibrotic effects of sGC stimulators. Ann Rheum Dis. 77:459–463

Liang R, Šumová B, Cordazzo C, Mallano T, Zhang Y, Wohlfahrt T, Dees C, Ramming A, Krasowska D, Michalska-Jakubus M, Distler O, Schett G, Šenolt L, Distler JH (2017) The transcription factor GLI2 as a downstream mediator of Transforming Growth Factor-β induced fibroblast activation in SSc. Ann Rheum Dis. 76(4): 756-764

Huang J, Maier C, Zhang Y, Soare A, Dees C, Beyer C, Harre U, Chen C-W, Distler O, Schett G, Wollin L, Distler JH (2017) Nintedanib macrophage activation and ameliorates vascular and fibrotic manifestations in the Fra2 mouse model of systemic sclerosis. Ann Rheum Dis. 76: 1941–1948

Rauber S, Luber M, Weber S, Maul L, Soare A, Wohlfahrt T, Lin N-Y, Dietel K, Bozec A, Herrmann M, Kaplan MH, Weigmann B, Zaiss M, Fearon U, Veale DJ, Canete JD, Distler O, Rivallese F, Pitzalis C, Neurath MF, McKenzie ANJ, Wirtz S, Schett G, Distler JH, Ramming A (2017) Resolution of inflammation by interleukin 9 producing type 2 innate lymphoid cells. Nat Med. 23: 938-944

Zhang Y, Liang R, Chen C-W, Mallano T, Dees C, Distler A, Reich A, Bergmann C, Ramming A, Gelse K, Milenz D, Distler O, Schett G, Distler JH (2017) JAK1-dependent transphosphorylation of JAK2 limits the anti-fibrotic effects of selective JAK2 inhibitors upon long-term treatment. Ann Rheum Dis. 76: 1467-1475

Maier C, Ramming A, Bergmann C, Weinkam R, Kittan N, Schett G, Distler JH, Beyer C (2017) Inhibition of phosphodiesterase 4 (PDE4) reduces dermal fibrosis by interfering with the release of interleukin-6 from M2 macrophages. Ann Rheum Dis. 76: 1133-1141

Chen CW, Beyer C, Liu J, Maier C, Li C, Trinh-Minh T, Xu X, Cole SH, Hsieh MH, Ng N, Althage A, Meeusen S, Pan S, Svensson EC, Seidel HM, Schett G, Gergely P, Harris JL, Distler JH (2017) Pharmacological inhibition of porcupine induces regression of experimental skin fibrosis by targeting Wnt signalling. Ann Rheum Dis. 76: 773–778

Palumbo-Zerr K, Soare A, Zerr P, Liebl A, Mancuso R, Tomcik M, Sumova B, Dees C, Chen CW, Wohlfahrt T, Mallano T, Distler A, Ramming A, Gelse K, Mihai C, Distler O, Schett G, Distler JH (2016) Composition of TWIST1 dimers regulates fibroblast activation and tissue fibrosis. Ann Rheum Dis. 76(1): 244-251

Lin NY, Distler A, Beyer C, Philipi-Schöbinger A, Breda S, Dees C, Stock M, Tomcik M, Niemeier A, Dell'Accio F, Gelse K, Mattson MP, Schett G, Distler JH (2016) Inhibition of Notch1 promotes hedgehog signalling in a HES1-dependent manner in chondrocytes and exacerbates experimental osteoarthritis. Ann Rheum Dis. 75: 2037-2044

A65 - Final Report

01/04/2016 - 31/03/2019

Tolerizing potential of human dendritic cell subpopulations

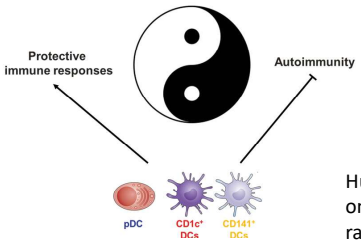
Prof. Dr. Diana Dudziak, Department of Dermatology

Dendritic cells (DCs) play a major role in the maintenance of tolerance. Expression profiling of DC subsets revealed that lympho-hematopoietic organs have only a minor influence on DC ontogeny and the resulting DC subtype signature in the steady state. Strikingly, isolated thymic DCs displayed a tolerogenic phenotype upon pathogenic stimulation. We are aiming to investigate this tolerizing potential in thymic DC subsets on functional and epigenetic level.

In our preliminary data, we sorted DC subsets from blood, spleen, and thymus and stimulated the isolated DCs with Toll like receptor (TLR) ligands. After culture, we measured the concentration of secreted cytokines and chemokines by CBA assay from collected supernatants. Strikingly, we found that the cellular surface expression profile did not reflect the secreted cytokines and chemokines as thymic DCs expressed all costimulatory molecules expected to be there upon pathogenic stimulation. However, the amount of several cytokines was strongly reduced in the supernatants of thymic DCs compared to blood or splenic DCs. Most importantly, we found that the production of the TH1 polarizing cytokine IL-12 was almost completely blocked in the thymus, whereas blood and splenic DCs produced comparable amounts of it. We hypothesize that DCs in the thymus have a tolerogenic potential, inhibiting the pre-activation of thymocytes even in the case of a potential infection. We are interested to understand this tolerizing potential of human thymic DCs as this immunoprivileged site might harbor important aspects also for understanding of tumor development.

Results in Aim 1: Determination of functional differences between blood and thymic DCs

To strengthen our preliminary findings we performed polarization and proliferation assays in a co-culture of isolated and activated thymic and blood DCs with HLA-mismatched naïve CD4+ peripheral blood T cells. Cell-sorted DCs from these tissues were stimulated with TLR ligands. After 24 hrs, the cells were co-cultured with purified allogeneic blood CD4+ T cells. Intracellular FACS analyses revealed that thymic DCs are inhibited in driving TH1 differentiation, while the differentiation into TH2 and TH17 was unaltered compared to blood DCs. We currently investigate CD8+ T cell driven and NK responses.



Human DC subpopulations and their role in tolerance and immunity.

Results in Aim 2: Identification of epigenetic modifications in steady-state and activated thymic and blood DC subpopulations

Thymic DCs might be influenced by either tissue factors or differential activity of regulatory components (e.g. transcriptions factors). Investigating the expression of NFκB and NFκB regulating factors we found only slight differences between thymic and blood DCs. Therefore, we measured the phosphorylation of signaling molecules upon stimulation with TLR ligands of sorted blood and thymic DCs. Notably, we found a differential DC specific phosphorylation profile in that NFκB p65 and p38 MAPK were stronger and longer phosphorylated in R848-stimulated blood DCs compared to thymic DCs. Those data are of specific importance, as we needed more evidence about potential regulatory mechanisms in thymic DCs. Based on our findings, we have sorted non-activated and TLR-activated DC subsets and performed Nanostring analyses. Epigenetic profiling, including ATAC sequencing combined with RNA-Seq analyses will be performed next.

Contact:
Prof. Dr. Dudziak
phone: +49 9131 85 39346
e-mail: diana.dudziak@uk-erlangen.de

Prof. Dr. Dudziak

Invited lectures

ImmunoBenin 1 week Immunology course, Benin, Antigen presenting cells: Dendritic cells and others, 03-11.11.2019

China, Understanding dendritic cell subset functions for the development of future cancer therapies IUIS, Beijing, 21st Oct 2019

Annual Meeting of the Autumn School of Immunology, Merseburg, 'How dendritic cells (interact and) activate T cells', 16.10.2019

Erlangen, 10 years of SEON, Immunonkologische Ansätze in der Nanomedizin - Aspects of the Emerging Fields Initiative - BIG-THERA, 18.09.2019

49th Annual Meeting of the German Society of Immunology, Munich workshop chair, 10.-13-09.2019

Paris, Antigen processing and presentation (APP10) EMBO, Assembly of the immunoproteasome in cDC2 allows for cross-presentation of antigens under stimulatory conditions in vivo, 29.05.-02.06.2019

Awards

Elected member of the review board (Fachkollegium) of the German Research Foundation (DFG), 2019

Coordinator and speaker of the Emerging Fields Initiative of the Friedrich-Alexander-University Erlangen-Nürnberg 'Integrative ‚Big Data Modeling' for the development of novel therapeutic approaches for breast cancer - BIG-THERA – 2017-2020

Elected member of the executive board of the German Society of Immunology (DGfI), since 2017

Speaker and organizer of the annual meeting of the 'Dendritic Cell Study Group (AKDC)', Budenheim (near Mainz), since 2017

Publications during funding period

Amon L., Lehmann CHK, Baranska A, Schoen J, Heger L, Dudziak, D (2019) Transcriptional control of dendritic cell development and functions. Int Rev Cell Mol Biol. (349):55-151

Dudeck J, Froebel J, Kotrba J, Lehmann CHK, Dudziak D, Speier S, Nedospasov SA, Schraven B, Dudeck A (2019) Engulfment of mast cell secretory granules on skin inflammation boosts dendritic cell migration and priming efficiency. J Allergy Clin Immunol. 143(5):1849-1864

Fischer A, Abdollahi-Roodsaz S, Böhm C, Niederreiter B, Meyer B, Yau ACY, Lönnblom E, Joosten LAB, Koenders M, Lehmann CHK, Dudziak D, Krönke G, Holmdahl R, Steiner G (2018) The involvement of Toll-like receptor 9 in the pathogenesis of erosive autoimmune arthritis. J Cell Mol Med. 22(9):4399-4409

Heger L, Balk S, Lühr JJ, Heidkamp GF, Lehmann CHK, Hatscher L, Purbojo A, Hartmann A, Garcia-Martin F, Nishimura S-I, Cesjnevar R, Nimmerjahn F, Dudziak D (2018) CLEC10A is a specific marker for human CD1c+ DCs and enhances their Toll-like receptor 7/8 induced cytokine secretion. Front Immunol. 9:744

Busch L, Mougiakakos D, Büttner-Herold M, Müller MJ, Volmer DA, Bach C, Fabri M, Bittenbring JT, Neumann F, Boxhammer R, Nolting J, Bisht S, Böttcher M, Jitschin S, Hoffmann MH, Balzer H, Beier F, Gezer D, Dudziak D, Gelse K, Hennig FF, Pallasch CP, Spriewald B, Mackensen A, Bruns H (2018) Lenalidomide enhances MOR202-dependent macrophage-mediated effector functions via the vitamin D pathway. Leukemia. doi: 10.1038/s41375-018-0114-0

Messlinger H, Sebald H, Heger L, Dudziak D, Bogdan C, Schleicher U (2018) Monocyte-Derived Signals Activate Human Natural Killer Cells in Response to Leishmania Parasites. Front Immunol. 9:24.

Friede ME, Leibelt S, Dudziak D, Steinle A (2018) Select Clr-g Expression on Activated Dendritic Cells Facilitates Cognate Interaction with a Minor Subset of Splenic NK Cells Expressing the Inhibitory Nkrp1g Receptor. J Immunol. 200(3):983-996.

Schneppenheim J, Loock AC, Hüttl S, Schweizer M, Lüllmann-Rauch R, Oberg HH, Arnold P, Lehmann CHK, Dudziak D, Kabelitz D, Lucius R, Lennon-Duménil AM, Saftig P, Schröder B (2017) The influence of MHCII on B cell defects 1 induced by invariant chain/CD74 N2 terminal fragments. J Immunol. 199(1):172-185

Lehmann CHK, Baranska A, Heidkamp GF, Heger L, Neubert K, Lühr JJ, Hoffmann A, Reimer K, Brückner C, Beck S, Seeling M, Kießling M, Soulat D, Krug A, Ravetch JV, Leusen JHW, Nimmerjahn F*, Dudziak D* (2017) DC subset specific induction of T cell responses upon antigen uptake via Fcγ receptors in vivo. J Exp Med. 214(5):1509-1528

Mokada-Gopal L, Boeser A, Lehmann CHK, Drepper F, Dudziak D, Warscheid B, Voehringer D (2017) Identification of Novel STAT6-Regulated Proteins in Mouse B Cells by Comparative Transcriptome and Proteome Analysis. J Immunol. 198(9):3737-3745.

Gerstner S, Köhler W, Heidkamp G, Purbojo A, Uchida S, Ekici AB, Heger L, Luetke-Eversloh M, Schubert R, Bader P, Klingebiel T, Koehl U, Mackensen A, Romagnani C, Cesnjevar R, Dudziak D, Ullrich E (2016) Specific phenotype and function of CD56-expressing innate immune cell subsets in human thymus. J Leukoc Biol. 100(6):1297-1310

Heidkamp GF*, Sander J*, Lehmann CH, Heger L, Eissing N, Baranska A, Lühr JJ, Hoffmann A, Reimer K, Lux A, Söder S, Hartmann A, Zenk J, Ulas T, McGovern N, Alexiou C, Spriewald B, Mackensen A, Schuler G, Schauf B, Forster A, Repp R, Fasching PA, Purbojo A, Cesnjevar R, Ullrich E, Ginhoux F, Schlitzer A, Nimmerjahn F, Schultze JL*, Dudziak D* (2016) Human lymphoid organ dendritic cell identity is predominantly dictated by ontogeny, not tissue microenvironment. Science Immunol 1-17, DOI: 10.1126/sciimmunol aai7677

A66 - Final Report

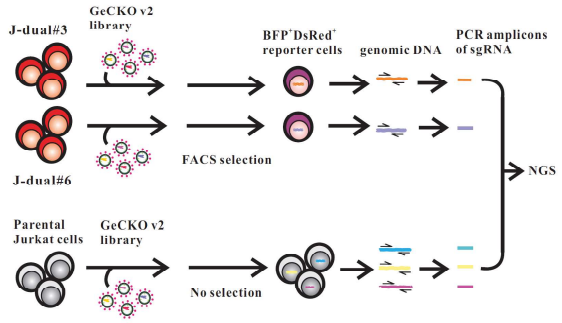
01/07/2016 - 30/06/2019

Genome wide CRISPR/Cas9 knockout for the identification of antiviral cellular restriction factors

Prof. Dr. Armin Ensser, Institute of Clinical and Molecular Virology

The project focused on cellular factors that restrict herpesviruses and/or limit the growth of tumor cells transformed by human gammaherpesviruses. These factors represent primary therapeutic targets. We employed a two-pronged, unbiased approach at identifying such restriction factors using the powerful CRISPR/Cas9 knockout technology. One system is targeting each human gene with several independent constructs for knockout, the other system is capable of activating the promoter of each human gene.

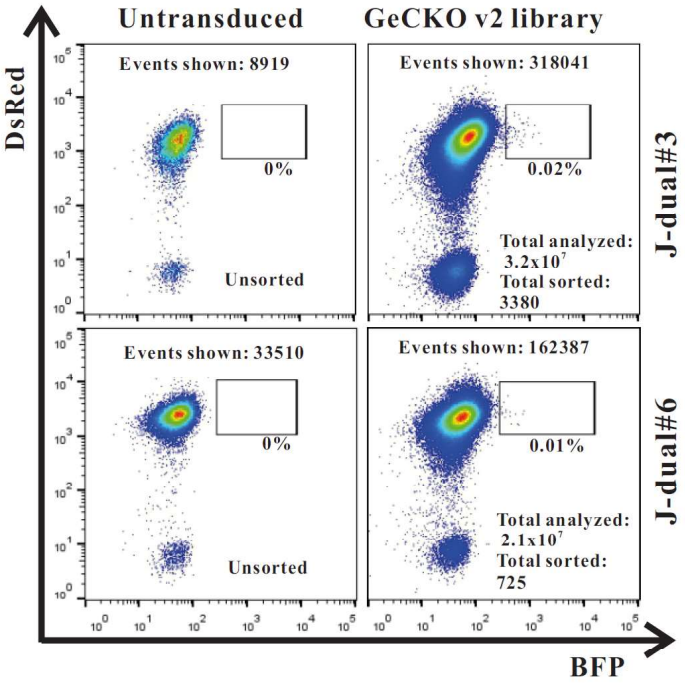
Viruses, like other intracellular parasites, must evade the actions of the host cell's innate immune response, and often devote a substantial portion of their coding capacity to counteract these cellular restriction factors. The systematic and unbiased approach at identifying cellular restriction factors of DNA viruses uses the powerful CRISPR/Cas9 knockout and SAM technology. The projects major objectives are (1) performing complementary, unbiased CRISPR/Cas9 based screens for the identification of novel candidate cellular factors restricting DNA viruses and in particular Gammaherpesviruses, and factors restricting growth of Gammaherpesvirus-transformed cells; this data from objective (1) will also represent a valuable resource on their own that can be tapped into for future research projects. Objective (2) is the verification of a subset of these cellular candidate restriction factors, that are selected based on novelty and effect strength, which is followed by the (3) identification of the viral proteins that are the targets of the cellular restriction factors and (4) the elucidation of the mechanism.



Genome-wide CRISPR/Cas screen for Rev-independent expression of unspliced HIV1 transcripts, outline of screening strategy (with permission from Han Xiao et al, 2020 submitted).

After performing first genome wide screening experiments on KSHV infected SLK target cells, we found the initial screens were limited by the FACS-sorting rate of large epithelial cells, such as SLK(Caki) or HeLa, which allowed to separate ~1500 cells per second, and would have resulted in sort times exceeding 20 hours for a typical sample of 50-100 million cells. Therefore, we constructed recombinant KSHV Bac16, Bac16RGB and HVS, expressing a murine H-2Kk surface marker that allows magnetic (pre-) sorting of virus infected cells. This can then be followed by FACS sorting of GFP and GFP/BFP populations. The synergistic activation mediator (SAM) system was functionally verified and established as a complementary approach to validate the sgRNA ko-screen. Its genome-wide sgRNA2.0 library of >70.000 plasmids is now available and the components of the SAM system, NLS-dCas9-VP64 and helper transactivator MS2-p65-HSF1 were tested in model cell lines (e.g. SLK(Caki), HeLa). Furthermore, an improved version of the genome-wide sgRNA library in the pLentiCRISPRv2 vector was obtained and amplified. This "Brunello-Library" consisting of 4 sgRNA per cellular gene and 1000 non-targeting control sgRNAs (total 77.441 constructs) and is more efficient due to its optimized algorithm for sgRNA design, resulting in a lower false discovery rate (FDR), and the lower complexity requires 1/3 less cells to be transduced, and consequently, less sorting time for selection of enriched or depleted cells. In summary, the two complementary screens already helped us to identify targets with increased confidence via the respective opposite ranking in knockout vs. SAM screens, ensuring that we can now focus on relevant genes.

Prof. Dr. Ensser



Flow cytometric analysis of J-dual#3 and J-dual#6 reporter cells without transduction and four days after transduction by the GeCKO v2 lentiviral vector library (with permission from Han Xiao et al, 2020 submitted).

The screening platform forms the basis of a DFG grant application related to the identification of factors restricting mammalian 1 orthobornavirus in human epithelial and neuronal cells; this DFG application EN423/6-1 is currently under revision to address the reviewer's comments. The Brunello Library was furthermore used to screen for factors restricting CMV reactivation in undifferentiated monocytes; to search for factors restricting replication and glycoprotein-dependent entry of RRV, the model virus of KSHV, in infected human cells; to identify factors that modify Human immunodeficiency virus Rev protein-independent nuclear RNA export into the cytoplasm. Corresponding data is now being functionally verified and will result in further publications.

Contact:
Prof. Dr. Ensser
phone: +49 9131 85 22104
e-mail: armin.ensser@fau.de

Publications during funding period

Full F, van Gent M, Sparrer KMJ, Chiang C, Zurenski MA, Scherer M, Brockmeyer NH, Heinzerling L, Sturzl M, Korn K, Stamminger T, Ensser A, Gack MU (2019) Centrosomal protein TRIM43 restricts herpesvirus infection by regulating nuclear lamina integrity. Nat Microbiol 4: 164-176. Epub 28.11.2018

Ensser A, Grosskopf AK, Matz-Rensing K, Roos C, Hahn AS (2018) Isolation and sequence analysis of a novel rhesus macaque foamy virus isolate with a serotype-1-like env. Arch Virol 163: 2507-2512

Grosskopf AK, Ensser A, Neipel F, Jungnickl D, Schlagowski S, Desrosiers RC, Hahn AS (2018) A conserved Eph family receptor-binding motif on the gH/gL complex of Kaposi's sarcoma-associated herpesvirus and rhesus monkey rhadinovirus. PLoS Pathog 14: e1006912

Proff J, Brey CU, Ensser A, Holter W, Lehner M (2018) Turning the tables on cytomegalovirus: targeting viral Fc receptors by CARs containing mutated CH2-CH3 IgG spacer domains. J Transl Med 16: 26

Ensser A (2017) Genome Sequence of the Alcelaphine Gammaherpesvirus 1 Attenuated Laboratory Strain WC11. Genome Announc 5: e01219-17; doi: 10.1128/genomeA.01219-17

Full F, Hahn AS, Grosskopf AK, Ensser A (2017) Gammaherpesviral Tegument Proteins, PML-Nuclear Bodies and the Ubiquitin-Proteasome System. Viruses 9: 308; doi: 10.3390/v9100308

A67 - Final Report

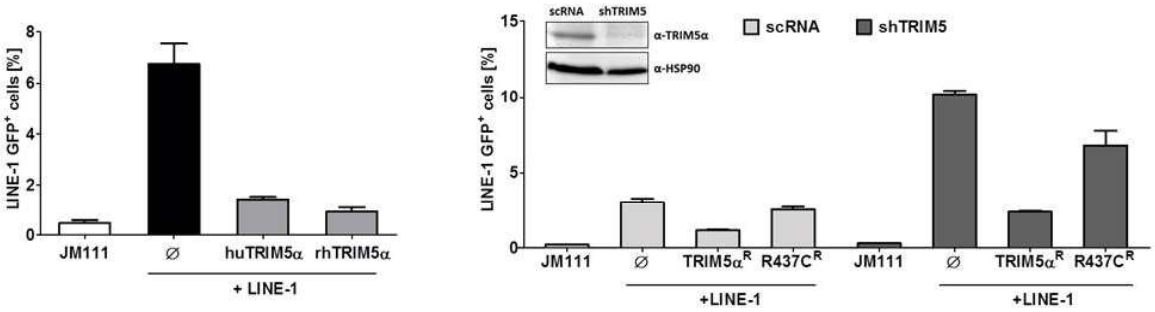
01/02/2016 - 31/01/2019

Analysis of the TRIM5alpha-mediated block to LINE-1 retroelements

Prof. Dr. Thomas Gramberg, Institute of Clinical and Molecular Virology

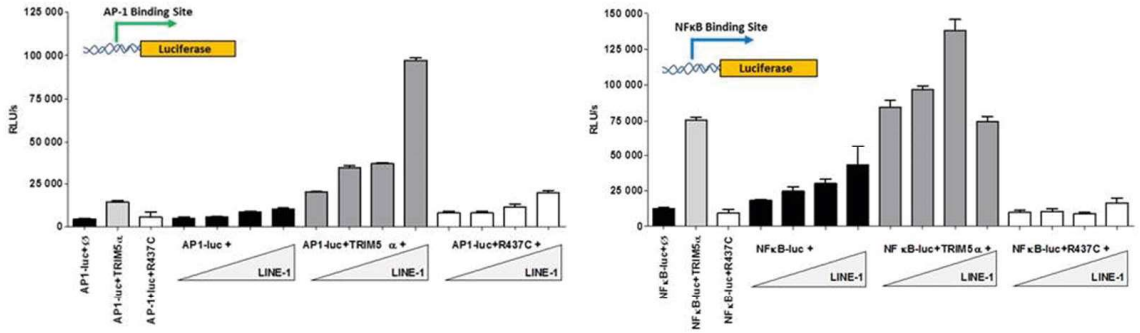
LINE-1 is the only autonomously active retrotransposon in humans. LINE-1 retrotransposition has been shown to cause various genetic disorders. Thus, it is important to control LINE-1 activity to maintain genome integrity. We found that the retroviral restriction factor TRIM5α also inhibits LINE-1 elements. In this study, we will determine the features of TRIM5α important for this block. We will analyze the mechanism of LINE-1 inhibition and ask if other mobile elements are restricted as well.

Initially, we demonstrated that the retroviral restriction factor human TRIM5α reduces the retrotransposition frequencies of LINE-1 reporter elements. In addition, we were able to establish stable TRIM5a knockdown cells and found that the knockdown of endogenous TRIM5α enhanced LINE-1 retrotransposition. By establishing a digital droplet PCR protocol we saw that the number of LINE-1 integrates is significantly reduced in the presence of TRIM5α. To identify regions within TRIM5α important for LINE-1 restriction, we analyzed naturally occurring TRIM5α SNPs and TRIM5α deletion mutants in LINE-GFP reporter assays. We identified a single SNP located within the SPRY domain of TRIM5α, which directly interacts with retroviral capsids, that causes a complete loss of LINE-1 restriction. To confirm the importance of the SPRY domain and to determine the role of the B-Box domain, which is important for multimerization of TRIM5α, in LINE-1 restriction, we analyzed the retrotransposition efficiency of LINE-GFP in the presence of various SAMHD1 mutants. We were able to confirm the importance of the SPRY domain for LINE-1 restriction and found that changing critical amino acids within the B-box strongly reduced TRIM5α activity against LINE-1. This suggests that, similar to HIV restriction, multimerization of TRIM5α via its B-Box is necessary for a direct interaction of TRIM5a with LINE-1 via its SPRY domain. In addition, we found that TRIM5α directly interacts with LINE-1 ribonucleoprotein complexes upon coexpression and both proteins, TRIM5α and LINE-1 ORF1p, colocalize in the cytoplasm suggesting a direct interaction of LINE-1 ribonucleoprotein particles and TRIM5α in the cytoplasm.



TRIM5α restricts LINE-1. (Left) 293Ts were transfected with LINE-GFP together with human or rhesus TRIM5a. (Right) 293Ts expressing shRNA targeting TRIM5α or control shRNAs were transfected with LINE-GFP and shRNA-resistant TRIM5a (TRIM5aR). Retrotransposition was quantified 5 days later by FACS.

Prof. Dr. Gramberg



TRIM5α induces NF-kB and AP-1 signaling in response to LINE-1. 293Ts were transfected with NF-kB or AP-1-dependent luciferase reporter gene, TRIM5am and LINE-1. AP-1 or NF-kB activity was quantified 2 days later by luciferase assay.

Since the interaction of TRIM5a with retroviral capsids has been shown to induce AP-1 and NfkB signaling, we asked whether the TRIM5α-mediated induction of AP-1 and NF-kB might also play a role in LINE-1 restriction. Indeed, we found that the replication of LINE-1 GFP is potently restricted by overexpression of constitutively active mutants of the AP-1 signaling pathway. In AP-1 and NF-kB-luciferase reporter assays, TRIM5 expression led to NF-kB and AP-1 activation. Interestingly, the TRIM5α-mediated AP-1 and NF-kB signal in the presence of LINE-1 was enhanced, suggesting that TRIM5α induces inhibitory AP-1 signaling upon binding to LINE-1. Conclusively, in LINE-1 promoter reporter assays, we found that the induction of AP-1 negatively regulates LINE-1 promoter activity. Together, our results suggest that multimeric TRIM5α senses LINE-1 protein complexes in the cytoplasm and downregulates LINE-1 promoter activity in a negative feedback loop by activating AP-1 signaling. Therefore, we show that TRIM5α is sensing and limiting LINE-1 retrotransposition and is thereby contributing to genome integrity.

Contact:
Prof. Dr. Gramberg
phone: +49 9131 85 36481
e-mail: thomas.gramberg@fau.de

Invited lectures

GfV Jahrestagung, 20.03.2019, Düsseldorf, “Human TRIM5α senses and blocks the replication of LINE-1 retroelements”

Publications during funding period

none

A68 - Final Report

16/06/2016 - 15/06/2019

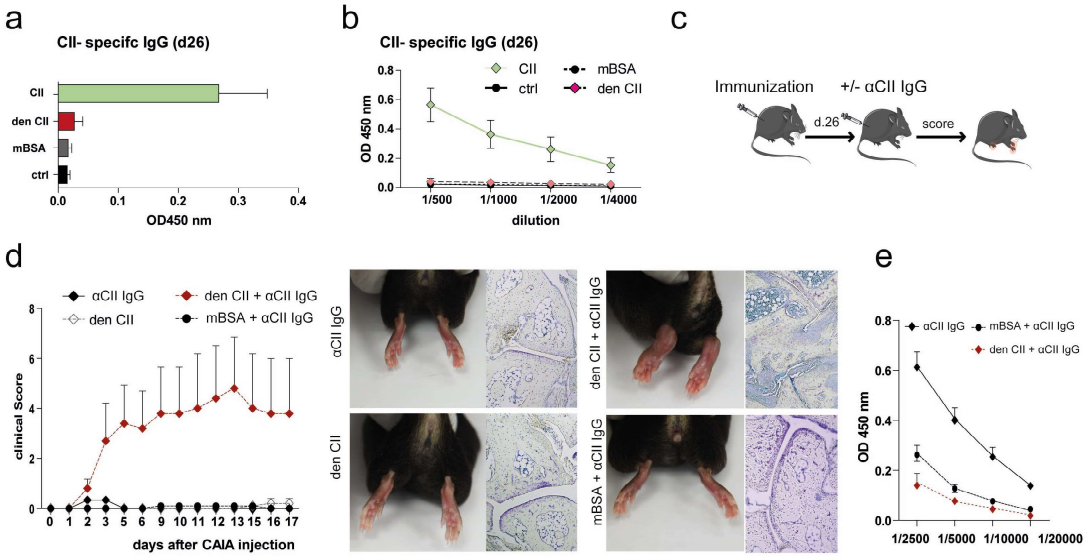
Analysis of the role of the IL-23/Th17 axis during the control of antibody activity in rheumatoid arthritis

Prof. Dr. Gerhard Krönke, Department of Medicine 3 – Rheumatology and Immunology
Prof. Dr. Falk Nimmerjahn, Division of Genetics

In our project we were analyzing the role of the IL-23/TH17 axis during the pathogenesis of rheumatoid arthritis (RA). We have shown that TH17 cells control of the intrinsic inflammatory activity of autoantibodies during onset of autoimmune arthritis via regulation of the expression of glycosyltransferases in plasma cells. Recent data additionally show that TH17 cells also control the onset of arthritis by licensing the tissue for autoantibody-induced inflammation.

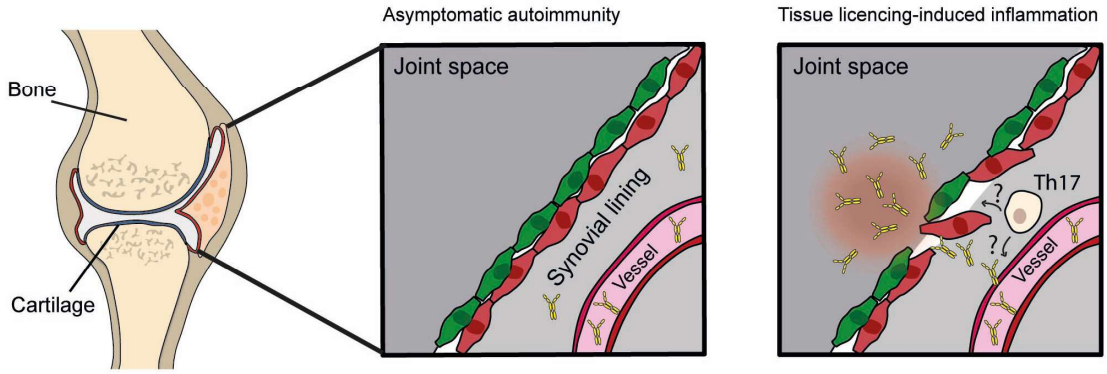
We previously identified the IL-23/TH17 axis as important modulator of the inflammatory activity of autoantibodies during rheumatoid arthritis (RA). TH17 cells critically contributed to the initial production of pro-inflammatory and arthritogenic IgG before onset of inflammation. Here, we identified TH17 cells in secondary lymphatic organs that displayed a T follicular helper cell phenotype and entered germinal centers where they regulated the glycosyltransferase expression in newly differentiating plasma cells. This IL-23-dependent pathway determined the glycosylation profile of newly-produced autoantibodies.

Additional data show that collagen II (CII)-specific autoreactive TH17 cells also essentially control the onset of arthritis that develops upon passive transfer of CII-specific arthritogenic autoantibodies. Although



a, b) CII-specific IgG after immunization with CII, denatured CII (den CII) or mBSA. c) Experimental setting d) Collagen-induced arthritis (CIA) after immunization with den CII and mBSA in combination with transfer of αCII IgG. e) Titers of CII-specific IgG on day 42 after initial immunization.

Prof. Dr. Krönke Prof. Dr. Nimmerjahn



IL-23-dependent T cells license the joint for autoantibody-mediated inflammation via yet to be identified effector mechanisms.

underlying molecular mechanisms remain elusive, our preliminary findings suggest that autoreactive T cells “license” the joint for autoantibody-induced inflammation and that T cells and autoantibodies act in a synergistic and antigen-specific manner. These findings thus strongly suggest a role of specific T cell subsets in regulating the trafficking and activity of autoantibodies to and within the joint.

Contact:
Prof. Dr. Krönke
phone: +49 9131 85 43012
e-mail: gerhard.kroenke@uk-erlangen.de

Publications during funding period

Scholtysek C, Ipseiz N, Böhm C, Krishnacoumar B, Stenzel M, Czerwinski T, Palumbo-Zerr K, Rothe T, Weidner D, Klej A, Stoll C, Distler J, Tuckermann J, Herrmann M, Fabry B, Goldmann WH, Schett G, Krönke G (2018) NR4A1 Regulates Motility of Osteoclast Precursors and Serves as Target for the Modulation of Systemic Bone Turnover. J Bone Miner Res.;33(11): 2035-2047

Haberland K, Ackermann JA, Ipseiz N, Culemann S, Pracht K, Englbrecht M, Jäck HM, Schett G, Schuh W, Krönke G (2018) Eosinophils are not essential for maintenance of murine plasma cells in the bone marrow. Eur J Immunol. 48(5): 822-828

Rothe T, Ipseiz N, Faas M, Lang S, Perez-Branguli F, Metzger D, Ichinose H, Winner B, Schett G, Krönke G (2017) The Nuclear Receptor Nr4a1 Acts as a Microglia Rheostat and Serves as a Therapeutic Target in Autoimmune-Driven Central Nervous System Inflammation. J Immunol. 198(10): 3878-3885

Pfeifle R, Rothe R, Ipseiz N, Scherer HU, Culemann S, Harre U, Ackermann JA, Seefried M, Kleyer A, Uderhardt S, Haugg B, Hueber AJ, Daum P, Heidkamp GF, Ge C, Böhm S, Lux A, Schuh W, Magorivska I, Nandakumar KS, Lönnblom E, Becker C, Dudziak D, Wuhrer M, Rombouts Y Koeleman CA, Toes R, Winkler TH, Holmdahl R, Herrmann M, Blüml S, Nimmerjahn F, Schett G Krönke G (2017) Regulation of autoantibody activity by the IL-23/Th17 axis promotes the onset of autoimmune disease. Nat Immunol. 18(1): 104-113

A69 - Final Report

01/07/2016 - 30/06/2019

Contribution of ATM kinase and the DNA-damage response in innate immunity to infection

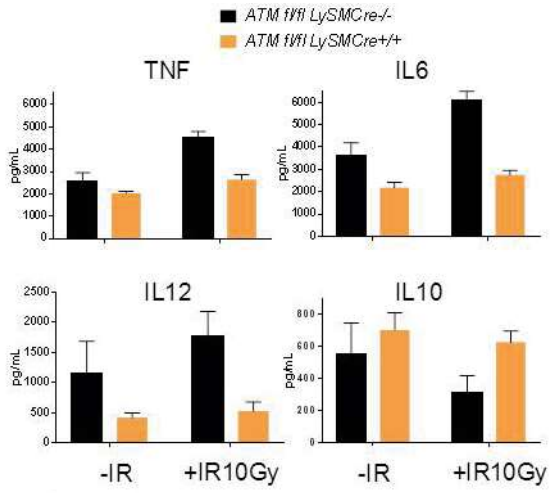
Prof. Dr. Roland Lang, Institute of Clinical Microbiology, Immunology and Hygiene

The DNA-damage response (DDR) requires the kinase ATM and is essential for the integrity of the host genome. We observed activation of the ATM kinase pathway in Toll-like receptor (TLR)-stimulated macrophages and a modulation of the inflammatory response by ATM-inhibition. Here, we carry out detailed studies to elucidate the molecular mechanisms and the consequences of ATM/DDR activation for the host response, protection and immunopathology during infection.

In this project, we employed conditional ATM knockout mice to investigate in vitro and in vivo how DNA damage response regulate the function of macrophages and dendritic cells (DC) upon encounter with microbial stimuli.

In a first set of in vitro experiments using bone marrow derived macrophages, we have observed that ionizing irradiation (IR) alters the balance of pro- and anti-inflammatory cytokine production in response to subsequent stimulation with LPS. TNF, IL-6 and IL-12 were significantly increased, whereas IL-10 was down-regulated. This effect of IR was completely dependent on ATM, as shown by Cre-mediated deletion macrophages (BMDM). Interestingly, non-irradiated ATM-deficient BMDM displayed moderately increased IL-10 and reduced proinflammatory LPS-induced cytokines compared to control BMDM, consistent with autochthonous activation of ATM by TLR4 activation. On the other hand, RNAseq analysis of genome-wide transcriptional changes revealed a surprisingly circumscript impact of IR and ATM on the response to TLR4 activation.

The transcription factor p53 is a canonical downstream target of ATM kinase. In irradiated macrophages, we found strong and dose-dependent phosphorylation of p53 after one hour, which was completely dependent on ATM. IR per se induced a moderate and transient upregulation of mRNA encoding IL-6 and TNF, with a peak between 1.5–3 hours. Pharmacological inhibitors of p53 and NFκB activation, but not of MAPK signaling, abrogated the



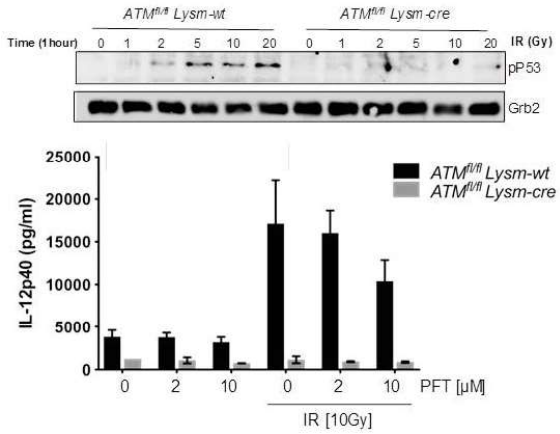
Irradiation alters the balance of cytokine production in response to the TLR4 ligand LPS. ATM-deficient and control bone marrow-derived macrophages (BMDM) were irradiated (10 Gy), followed by stimulation with LPS. Cytokines were measured from supernatants.

Prof. Dr. Lang

IR-induced cytokine expression. To further dissect the mechanisms of cross-regulation between DNA-damage response and TLR signaling in macrophages, we plan to employ conditional knockout mice for p53 and components of the NFκB pathway.

In vivo challenge with the TLR4-ligand LPS causes rapid systemic cytokine production, which can lead to organ damage and shock. Prior IR of mice with 2 Gy caused a significant reduction of TNF serum levels, whereas IL-6 production remained unaltered, suggesting that the consequences of DNA-damage induction are differentially regulated in vivo. The involvement of ATM kinase in immune responses to infection and inflammation is currently investigated using conditional knockout mice with ATM deletion in macrophages or DC.

Taken together, our results support the notion that the DNA damage response through ATM kinase exerts a significant regulatory effect on several innate cytokine responses to microbial danger. While macrophage activation status in vitro was clearly biased towards a more inflammatory response by DNA damage response, the resulting phenotypes in vivo were also compatible with an attenuation of inflammatory cytokine production by DNA damage and ATM. Therefore, in ongoing work we are investigating in more detail and breadth the effect of ATM-deficiency in murine infection and immunization models.



ATM-dependent p53 mediates IR-induced increase in cytokine production. (top) BMDM were irradiated with the indicated doses. Phosphorylation of p53 was determined after 1 h by Western blot. (bottom) Macrophages were treated as indicated with increasing doses of the p53 inhibitor Pifithrin-α.

Contact:
Prof. Dr. Lang
phone: +49 9131 85 22979
e-mail: roland.lang@uk-erlangen.de

Invited lectures

DGfI Autumn School Current Concepts in Immunology, October 14, 2019, Merseburg, Germany: Ontogeny and function of macrophages.

Institute of Medical Microbiology, Technical University Munich, April 8, 2019: Macrophage responses to mycobacteria – function and regulation

Publications during funding period

Hansen M, Peltier J, Killy B, Amin B, Bodendorfer B, Hartlova A, Uebel S, Bosmann M, Hofmann J, Buttner C, Ekici A B, Kuttke M, Franzyk H, Foged C, Beer-Hammer S, Schabbauer G, Trost M, and Lang R (2019) Macrophage Phosphoproteome Analysis Reveals MINCLE-dependent and -independent Mycobacterial Cord Factor Signaling. Mol Cell Proteomics 18: 669-685

A70 - Final Report

01/07/2016 - 30/06/2019

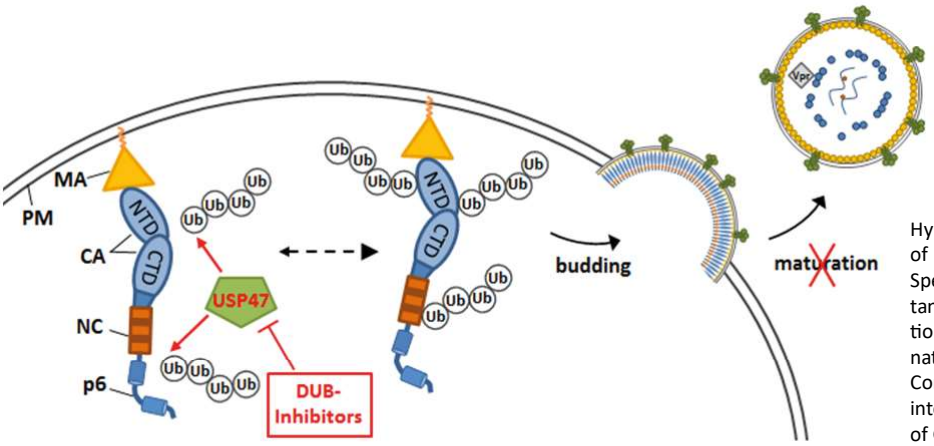
Novel targets for antiretroviral therapy – deubiquitinating enzymes regulate HIV-1 replication

Prof. Dr. Ulrich Schubert, Institute of Clinical and Molecular Virology

We found that certain deubiquitinating enzymes play an essential role in HIV-1 replication. In addition, we have been investigating the role of regulatory proteins in the interaction with the ubiquitin proteasome system. While Vpr is involved in HIV-associated fat metabolism diseases, Vpu directs the polyubiquitination of host cell-receptors. The small HIV-1 p6-Gag protein regulates membrane association and polyubiquitination of Gag and is specifically degraded by the insulin-degrading enzyme.

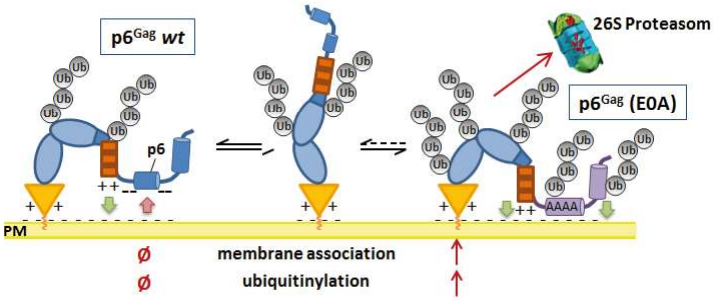
We were able to demonstrate for the first time that deubiquitinating enzymes (DUBs) are involved in HIV-1 replication by regulating Gag processing, and thus virus maturation and infectivity. As only certain DUB-inhibitors (DIs), which specifically inhibit the DUB USP47, have anti-retroviral activity, we hypothesized that USP47 plays an important role in HIV-1 replication. By performing loss of function analysis we could confirm that USP47 is crucial for the maintenance of the infectivity of HIV-1. Furthermore, we could show a complete block of virus replication by treatment of ex vivo cultivated human lymphoid tissue (HLT) with USP47-specific DIs. Thereby, virtually no toxicity was detected even at the highest concentrations used for treatment. Most strikingly, combinatory treatment of HLT with DIs and proteasome inhibitors in a concentration range, where both classes of inhibitors alone had no influence on virus replication, revealed a synergistic antiretroviral activity of inhibitors that act on both components of the UPS, proteasomes and DUBs.

To further unravel the role of the ubiquitin proteasome system (UPS) in HIV-1 replication we have been investigating the interaction of small HIV-1 proteins with the UPS. Currently, it is not clear of whether the ion channel activity of the regulatory HIV-1 Vpu protein is involved in the degradation of host cell receptors, like tetherin or CD4. However, together with our collaborators at the lab of Prof. Gerhard Thiel, TU Darmstadt, we were able to demonstrate that this activity is conserved throughout the evolution of HIV-1 and its ancestor SIVcpz. Concerning the HIV-1 regulatory protein Vpr we could show, together with the group of Dr. Balasubramanyam, Houston, USA, that Vpr, by interacting with the cellular DBB1, DCAF and Cul4A-ligase complex plays an important role in HIV-associated fatty liver diseases.



Hypothetical model: influence of DIs on HIV-1 replication. Specific DUBs play an important role in the HIV-1 replication as they regulate ubiquitination and processing of Gag. Consequently, DUB-inhibitors interfere with the maturation of Gag.

Prof. Dr. Schubert



Hypothetical model: the p6 mediated polyubiquitination of HIV-1 Gag. (E0A=p6 mutant lacking all negative charged amino acids)

The 52 aa HIV-1 p6 Gag protein does not only regulate the late steps in virus replication but also the polyubiquitination of Gag. Now we could demonstrate that the interaction of Gag with the plasma membrane and its subsequent polyubiquitination and access to DUBs, as well as the 26S proteasome, is regulated by the charge distribution within p6. Furthermore, we found that the p6 represents the first known viral substrate of an ubiquitously expressed cytosolic metalloendopeptidase, the insulin degrading enzyme (IDE). Thereby, p6 is approximately 100-fold more efficiently degraded by IDE than its eponymous substrate insulin. This phenomenon could be considered as one explanation for the significantly higher risk for type II diabetes in HIV-1 carriers. In addition, we were able to show that the degradation of p6 by the IDE is regulated by its N-terminus, a phenomenon which is specific for the pandemic HIV-1 group M isolates.

Contact:
Prof. Dr. Schubert
phone: +49 9131 85 26478
e-mail: ulrich.schubert@fau.de

Publications during funding period

Schmalen A, Karius-Fischer J, Rauch P, Setz C, Korn K, Henklein P, Fossen T, Schubert U (2018) The N-Terminus of the HIV-1 p6 Gag Protein Regulates Susceptibility to Degradation by IDE. Viruses 10(12): 710

Agarwal N, Iyer D, Gabbi C, Saha P, Patel SG, Mo Q, Chang B, Goswami B, Schubert U, Kopp JB, Lewis DE & Balasubramanyam A (2017) HIV-1 viral protein R (Vpr) induces fatty liver in mice via LXRα and PPARα dysregulation: implications for HIV-specific pathogenesis of NAFLD. Nature Scientific Reports 17;7(1): 13362

Setz C, Friedrich M, Rauch P, Fraedrich K, Matthaei A, Traxdorf M & Schubert U (2017) Inhibitors of Deubiquitinating Enzymes Block HIV-1 Replication and Augment the Presentation of Gag-Derived MHC-I Epitopes. Viruses 9(8): 222

Hahn F, Schmalen A, Setz C, Friedrich M, Schlößer S, Kölle J, Spranger R, Rauch P, Fraedrich K, Reif T, Karius-Fischer J, Balasubramanyam A, Henklein P, Fossen T & Schubert U (2017) Proteolysis of mature HIV-1 p6 Gag protein by the insulin-degrading enzyme (IDE) regulates virus replication in an Env-dependent manner. PLOS ONE 12(4): e0174254

Greiner T, Bolduan S, Hertel B, Groß C, Hamacher K, Schubert U, Moroni A & Thiel G (2016) Ion Channel Activity of Vpu Proteins Is Conserved throughout Evolution of HIV-1 and SIV. Viruses 8: 325

Friedrich M, Setz C, Hahn F, Matthaei A, Fraedrich K, Rauch P, Henklein P, Traxdorf M, Fossen T & Schubert U (2016) Glutamic Acid Residues HIV-1 p6 Regulate Virus Budding and Membrane Association of Gag. Viruses 25: 8(4)

A72 - Final Report

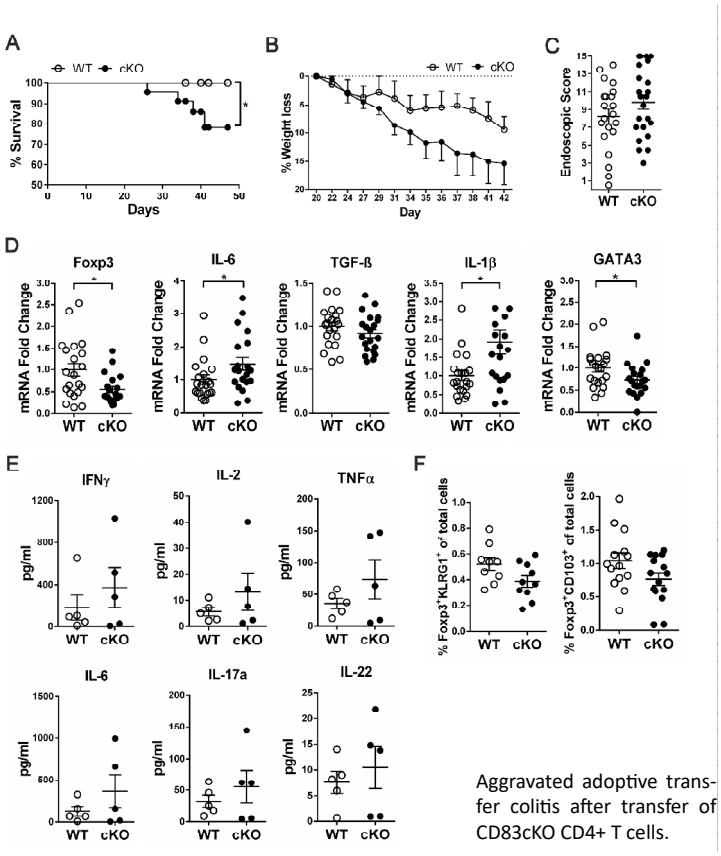
01/07/2016 - 30/06/2019

Targeted modulation of regulatory T cells and analyses of the underlying mechanisms

Prof. Dr. Alexander Steinkasserer, Department of Immune Modulation

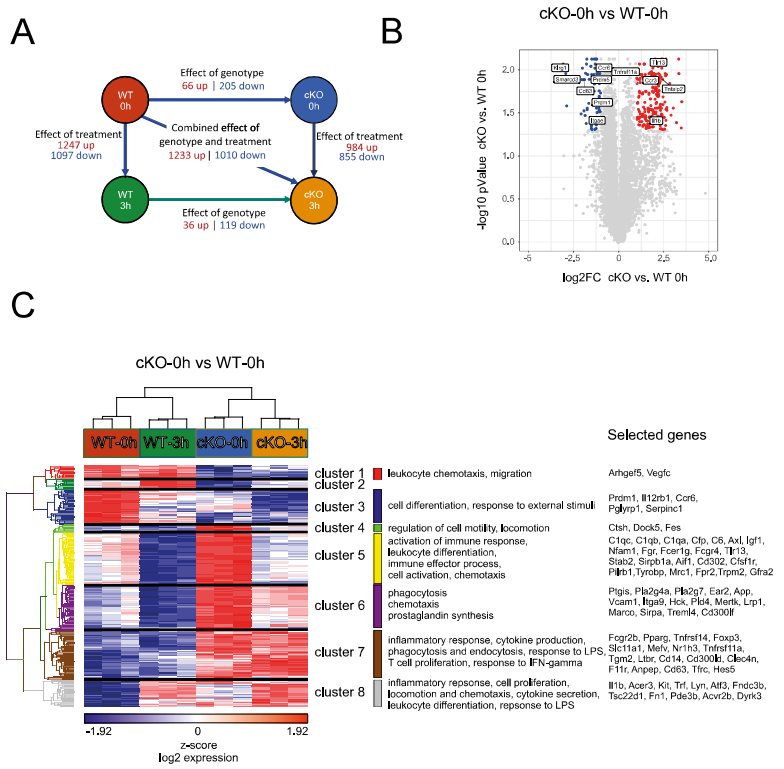
Regulatory T cells (Tregs) are crucial players to maintain immune homeostasis, to establish tolerance mechanisms and to prevent autoimmunity. Previously we showed that activated murine as well as human Tregs express the cell surface molecule CD83, indicating that this molecule is functionally important. Within the last year we elucidated the biological function of CD83 expression on Tregs using our Treg-specific CD83 conditional knockout (CKO) animals.

Upon activation, Tregs are transferred into an effector state expressing transcripts essential for their suppressive activity, migration and survival. However, how different intrinsic and environmental factors control differentiation is not completely understood. In order to investigate the specific role of CD83 exclusively on Tregs we generated conditional KO mice (Foxp3Cre CD83flox/flox) using the Cre-loxP system. Using these mice, we present for the first time data showing that Treg intrinsic expression of CD83 is essential for Treg differentiation upon activation. Interestingly, mice with Treg intrinsic CD83 deficiency are characterized by a pro-inflammatory phenotype. Furthermore, the loss of CD83 expression by Tregs leads to the downregulation of Treg specific differentiation markers and the induction of an inflammatory profile. Next, for in vivo analyses we used the EAE model, which is the best animal model to study the early inflammatory phase of multiple sclerosis (MS). These data revealed that animals, which do not express CD83 on their Tregs, show (i) an earlier and highly increased disease onset and (ii) a prolonged paralyses, indicating that the resolution of inflammation is critically impaired in CD83 CKO mice. These data clearly demonstrate that Treg specific CD83 CKO animals have a functional phenotype, supporting our hypothesis that CD83 is of critical importance for regulatory T cells. To further analyze these in vivo findings, we raised the question whether cKO Tregs can be equally expanded as WT Tregs upon activation in vitro. Thus, naïve CD4+CD25+CD62L+ T cells were cultured in the presence of IL-2 and anti-CD3/CD28 expansion beads and after 10 days, cKO Tregs show-



Aggravated adoptive transfer colitis after transfer of CD83cKO CD4+ T cells.

Prof. Dr. Steinkasserer



CD83 deletion on Tregs leads to highly differential gene expression. (A) Up- and downregulated genes between all 4 conditions. (B) Volcano plot comparing unstimulated cKO T cells versus WT Tregs. (C) Hierarchical clustering.

ed equal expansion rates as WT Tregs. However, on mRNA level we detected increased IFNγ levels in cKO Treg cells and a downregulation of GATA3 expression levels. Thus, we conclude that anti-CD3/CD28 and IL-2 stimulated cKO Tregs can be equally activated and expanded, however, cKO derived Tregs showed an altered pro-inflammatory cytokine pattern. To analyze if the suppressive capacity of cKO Tregs is impaired using an additional in vivo model, total CD4+ T cells from WT or cKO mice were isolated and transferred into RAG1-/- mice. Strikingly, a strongly increased mortality rate in RAG1-/- mice, and an increased weight loss was observed. In addition an increased clinical severity score with higher inflammation was observed in cKO cell transferred animals compared to WT controls. Analysis of mesenteric lymph nodes showed a significant lower infiltration of Tregs, whereas pro-inflammatory cytokine production was significantly increased. In contrast, significantly reduced GATA3 expression levels were detected in cKO animals. Flow cytometric analyses of mLNs revealed reduced KLRG1+ and CD103+ expression levels among the Foxp3+ T cell population in cKO cell transferred mice. This indicates a reduced number of terminal differentiated Tregs in the gastrointestinal tract of RAG1-/- mice after adoptive CD4+ T cell transfer from CD83cKO mice. Altogether, CD83 expression in Treg cells is an essential factor for the development and function of effector Treg cells upon activation. Since Treg cells play a crucial role in the maintenance of immune tolerance and thus prevention of autoimmune disorders, our findings are also clinically relevant.

Contact:
Prof. Dr. Steinkasserer
phone: +49 9131 85 36725
e-mail: alexander.steinkasserer@uk-erlangen.de

Publications during funding period

Döbbeler M, Koenig C, Krzyzak L, Seitz C, Wild A, Ulas T, Baßler K, Kopelyanskiy D, Butterhof A, Kuhnt C, Kreiser S, Stich L, Zinser E, Knippertz I, Wirtz S, Riegel C, Hoffmann P, Edinger M, Nitschke L, Winkler T, Schultze JL, Steinkasserer A, Lechmann M (2018) CD83 expression is essential for Treg cell differentiation and stability. JCI insight , 3(11). pii: 99712. doi: 10.1172/jci.insight.99712

A73 - Final Report

01/07/2016 - 30/09/2019

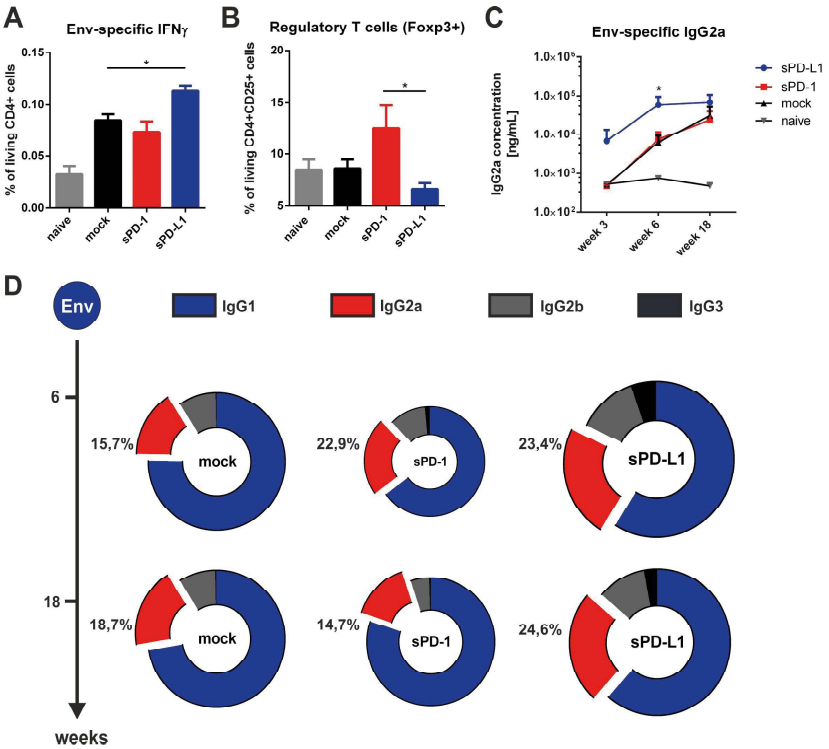
Checkpoint inhibitors as adjuvants for viral vaccines

Prof. Dr. Klaus Überla, Institute of Clinical and Molecular Virology

Checkpoint inhibition as a measure to improve T cell functions in the tumor microenvironment is an established platform of immunomodulation. During antigen-specific B cell responses PD-1 also controls germinal center reactions and affinity maturation and formation of long-lived plasma cells. By locally blocking immune checkpoints in a mouse model via co-electroporation of PD-1- and PD-L1-encoding DNA we observed modulatory effects on vaccine-induced immune responses against the surface proteins of HIV-1 and Influenza A, which could improve vaccine efficacies.

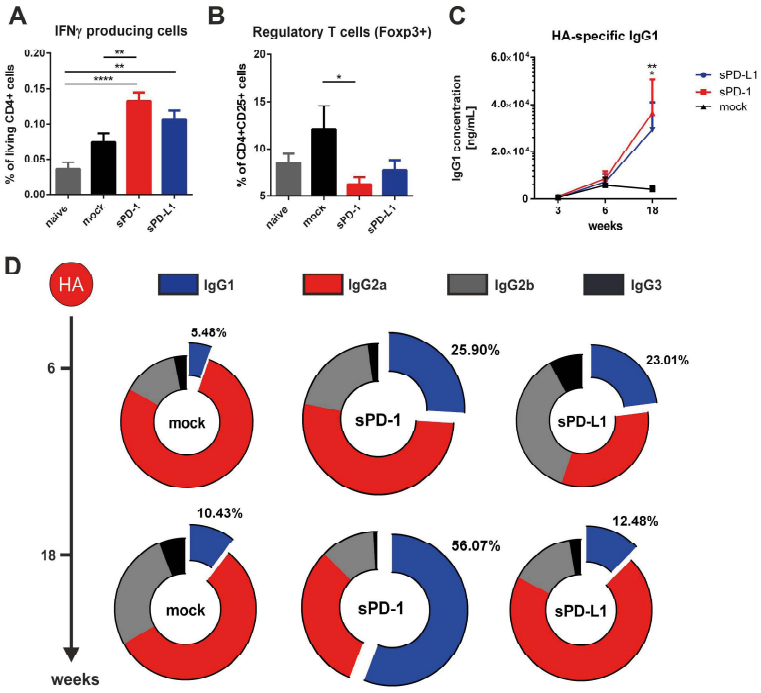
Checkpoint inhibition by monoclonal antibody administration induces anti-drug antibodies

Intraperitoneal application of monoclonal antibodies targeting PD-1 and its ligands after vaccination resulted in a spontaneous secretion of IL-4 and IL-5 compared to PBS-treated animals. Subsequently, a dosage-dependent elicitation of anti-drug antibodies was observed. This might interfere with the antigen-specific immunomodulation by checkpoint inhibitors. Therefore, we blocked immune checkpoints locally by co-electroporation of DNA vaccines encoding the active soluble ectodomain of PD-1 or PD-L1. With this blocking strategy, no systemic anti-drug effects were detected.



Soluble PD-L1 enhances Env-specific immune responses. Percentage of Env-specific CD4+ T cells producing IFNγ (A) and regulatory T cells (B) in the spleen of BALB/c mice. Env-specific IgG2a antibodies (C) and overall antibody response (D) over a time-period of 18 weeks.

Prof. Dr. Überla



Soluble PD-1 enhances HA-specific immune responses. Percentage of HA-specific CD4+ T cells producing IFNγ (A) and regulatory T cells (B) in the spleen of BALB/c mice. HA-specific IgG1 antibodies (C) and overall antibody response (D) over a time-period of 18 weeks.

HIV-1 Env-specific immune responses are modulated by PD-L1 encoding DNA

Co-electroporation of PD-L1-encoding DNA resulted in a significant upregulation of Env-specific IFN-producing CD4+ T cells compared to immunization with Env-DNA only (mock). Additionally, the frequency of regulatory T cells was downregulated in the PD-L1-treated animals, even 20 weeks after boosting. Finally, the Env-specific IgG2a antibody titers were substantially increased in PD-L1-treated mice with the overall antibody response shifting from a TH2-bias in mock- and PD-1-treated animals towards a more TH1/TH2 balanced subtype pattern in animals receiving PD-L1 treatment.

PD-1-encoding DNA modulates immune responses against Influenza HA

We also wanted to elucidate the modulatory capacity of the locally administered checkpoint molecules for the TH1-driven HA protein of Influenza. Interestingly, here co-electroporation of PD-1-encoding DNA resulted in a significantly higher frequency of HA-specific IFNgamma-secreting cells, a lower frequency of regulatory T cells and an increased elicitation of HA-specific IgG1 antibodies. The overall antibody responses was again more TH1/TH2 balanced compared to mock-treated animals. In addition, there was a trend towards higher levels of neutralizing antibodies in PD-1-treated animals.

In summary, these data indicate that checkpoint inhibition can modulate vaccine-induced cellular and humoral immune responses in an antigen-dependent manner.

Contact:
Prof. Dr. Überla
phone: +49 9131 85 23563
e-mail: klaus.ueberla@fau.de

Publications during funding period

Tannig P, Peter AS, Lapuente D, Klessing S, Damm D, Tenbusch M, Überla K, Temchura K (2020) Modulation of Vaccine-Induced HIV-1-Specific Immune Responses by Co-Electroporation of PD-L1 Encoding DNA. Vaccines 8: 27

A74 - Final Report

01/06/2016 - 31/05/2019

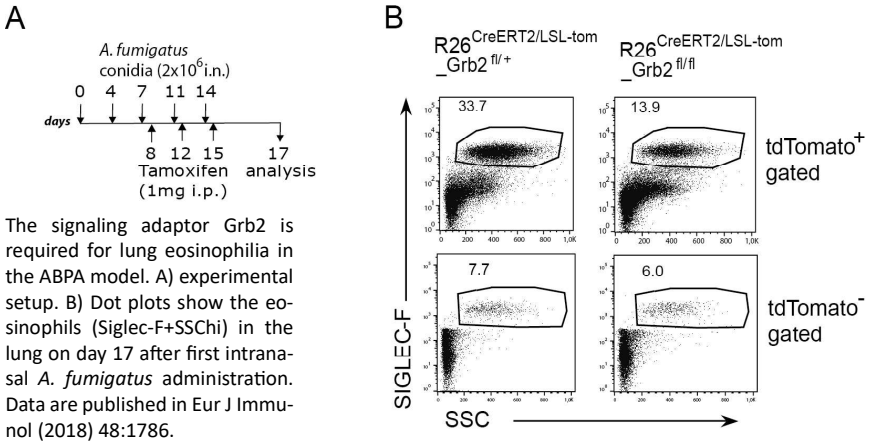
The Role of Eosinophils in Allergic Bronchopulmonary Aspergillosis

Prof. Dr. David Vöhringer, Department of Infection Biology
Prof. Dr. Sven Krappmann, Institute of Clinical Microbiology, Immunology and Hygiene

This interdisciplinary project targeted the intimate interplay of eosinophilic granulocytes with cells and cellular components of the fungal pathogen Aspergillus fumigatus in the context of allergic pulmonary aspergillosis (ABPA) which mainly affects asthma and cystic fibrosis patients. The main research questions we addressed were related to the contribution of eosinophils to immunopathology of ABPA and the activation of eosinophils by A. fumigatus infections in an established murine model of ABPA using recombinant mouse strains together with co-culturing experiments, aiming to shed light on the main host and fungal determinants triggering this complex allergic disease.

Using a mouse model with repetitive intranasal application of live or heat-inactivated *A. fumigatus* conidia, we could show that only live conidia elicited eosinophilia, expression of eosinophil-recruiting chemokines including eotaxins (CCL11 and CCL24), Th2 polarization, goblet cell hyperplasia and differentiation of alternatively activated macrophages in the lung. This response was entirely dependent on T cell-derived IL 4/IL-13 and slightly reduced in basophil-deficient mice. In vitro co-culture experiments further revealed that live but not heat-inactivated conidia stimulated eosinophils to release chemokines and cytokines as measured by Multiplex Luminex assays. The most strongly induced factors included IL-4, IL-13, IL-18, IL-23, IL-28 and TNF, MIP 1a, MIP-1b and MCP-1. Since eosinophil activation was dependent on viable conidia, we consider it likely that eosinophils respond either to secreted substances or cell wall components that only become accessible after germination. Interestingly, signaling through Toll-like receptors or C-type Lectin receptors apperaed not to be required for *A. fumigatus*-induced eosinophil activation since bone marrow-derived eosinophils from Myd88- and Card9-deficient mice showed an unaltered cytokine response.

We further investigated the role of the adaptor protein Grb2 in eosinophils for establishment of lung eosinophilia in the ABPA model. Grb2 mediates signaling downstream of the IL-5 receptor but was also reported to induce apoptosis so that the overall effects of Grb2 function in eosinophils remained unclear. Here, we used conditional Grb2-deficient mice crossed to R26CreERT2/LSL-tom mice so that by administration of tamoxifen Grb2 is deleted in tdTomato+ cells. These mice were subjected to the ABPA model and we observed that Grb2 was required for accumulation of eosinophils in the lung. These results were published last year (Willebrand et al., Eur J Immunol 48:1786).



The signaling adaptor Grb2 is required for lung eosinophilia in the ABPA model. A) experimental setup. B) Dot plots show the eosinophils (Siglec-F+SSChi) in the lung on day 17 after first intranasal *A. fumigatus* administration. Data are published in Eur J Immunol (2018) 48:1786.

To investigate fungal determinants of the host-pathogen interaction, in vitro experiments with different *A. fumigatus* mutant strains were performed. Previous studies had already demonstrated that fungal cell wall

Prof. Dr. Vöhringer Prof. Dr. Krappmann

The exopolysaccharide GAG and extracellular proteases impact the interaction of *A. fumigatus* with eosinophils. A) IL-4 concentrations of co-culture supernatants. Eosinophils were incubated with conidida of different fungal strains impaired in GAG formation with an MOI of 1:5 (E:T) for 7 and 12 h. B) IL-4 concentration of eosinophil culture, stimulated with culture supernatants of *A. fumigatus* wild-type, ΔprtT and reconstituted strains for 24 h.

carbohydrate structures are involved in the interaction with different immune cell types. We could show that the recently discovered fungal exopolysaccharide galactosaminogalactan (GAG), which is responsible for adhesion, also influences the activation of eosinophils: *A. fumigatus* strains lacking GAG or that exhibit an impaired deacetylation of GAG adhere less to eosinophils. Furthermore, the release of IL-4 and MIP1α (data not shown) is significantly reduced after co-cultivating eosinophils with these mutant strains. Whether this effect is based on direct recognition of GAG or an indirect effect due to impaired adherence and thus a reduced pattern recognition receptor binding of other PAMPs needs to be explored. Moreover, we became interested whether secreted proteases contribute to the eosinophil-fungus interaction. To address this issue, eosinophils were stimulated with culture supernatants of wild-type *A. fumigatus* and a strain which is deficient in the synthesis of extracellular proteases (ΔprtT). Whereas the supernatant of the wild-type strain induced secretion of IL-4, this effect could not be observed for the deletant, which indicates that also secreted fungal compounds, e.g. extracellular proteases, are able to activate eosinophils.

To identify novel fungal determinants in the eosinophil-fungus interaction, an *A. fumigatus* transcription factor (TF) deletion library was employed. Here we analyzed the fungal susceptibility towards the antimicrobial activity of eosinophils as well as the influence of each of the TF-encoding gene deletion on the capability to activate eosinophils. In further hit picking experiments, potential TF candidates are currently analyzed in more detail with respect to their influence on ABPA pathogenesis.

End of last year we submitted a DFG grant application based on our preliminary results from this IZKF-funded project to continue our research on the role of eosinophils in ABPA for the next three years.

Contact:
Prof. Dr. Vöhringer
phone: +49 9131 85 32735
e-mail: david.voehringer@uk-erlangen.de

Prof. Dr. Krappmann
phone: +49 9131 85 22580
e-mail: sven.krappmann@uk-erlangen.de

Invited lectures

Prof. S. Krappmann:
Göttinger Infectiological Colloquium, 8. May 2019, Institute of Medical Microbiology, University Medical Center Göttingen. Title: "Protecting the offspring - Linking developmental pathways of Aspergillus fumigatus to toxic compounds."

SFB 987 Seminar, 15. July 2019, Marburg. Title "Protecting the offspring - Linking developmental pathways of Aspergillus fumigatus to toxic compounds."

Publications during funding period

Willebrand R, Dietschmann A, Nitschke L, Krappmann S, Voehringer D (2018) Murine eosinophil development and allergic lung eosinophilia are largely dependent on the signaling adaptor GRB2. Eur J Immunol. 48: 1786-1795

A75 - Final Report

01/07/2016 - 30/06/2019

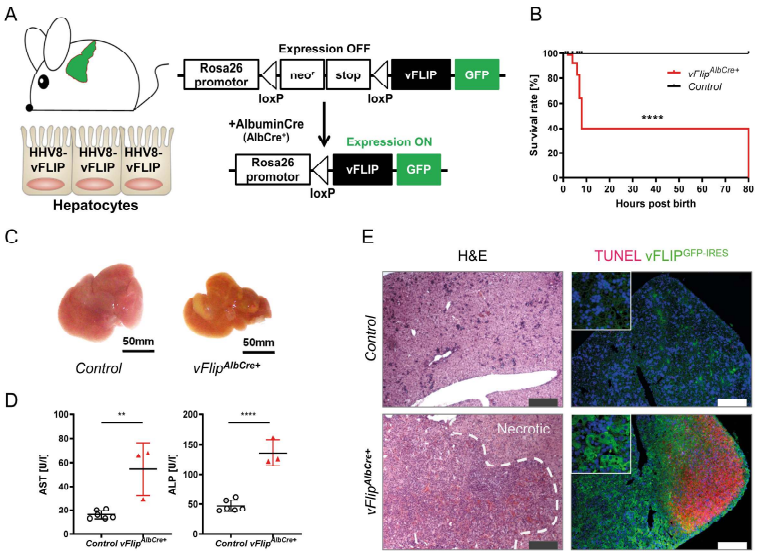
Role of MLKL-dependent programmed necrotic cell death in the pathogenesis of hepatitis

PD Dr. Dr. Claudia Günther, PD Dr. Dr. Stefan Wirtz,
Department of Medicine 1 – Gastroenterology, Pneumology and Endocrinology

In this project, we analyzed the role of the pseudokinase MLKL in liver diseases with a strong focus on autoimmune and infectious diseases. In order to investigate the contribution of MLKL to viral induced hepatitis, we established a new mouse model that is characterized by acute, cell death mediated liver dysfunction. Accordingly, we identified that transgenic expression of vFLIP, a viral Caspase-8 inhibitor causes severe liver injury that finally culminates in an early death of the mice.

Hepatocellular death plays a fundamental role in almost all hepatic diseases and thus, detailed knowledge about molecular mechanisms that mediate cell death responses in the liver is essential to advance therapeutic strategies. Previous concepts on cell death mechanisms have been recently challenged by the description of necroptosis, a form of programmed cell death mediated by the activation of RIP-kinases. The contribution of necroptosis to inflammatory liver diseases is controversial and particularly the role of mixed lineage kinase domain-like protein (MLKL), a recently identified key mediator of necroptosis, is largely unknown. The overall goal of this proposal was to identify how cell death is regulated during inflammatory liver injury. Based on our preliminary observations, we were particularly interested in the role of MLKL-dependent programmed necrosis in the pathogenesis of hepatitis characterized by hepatocellular necrosis.

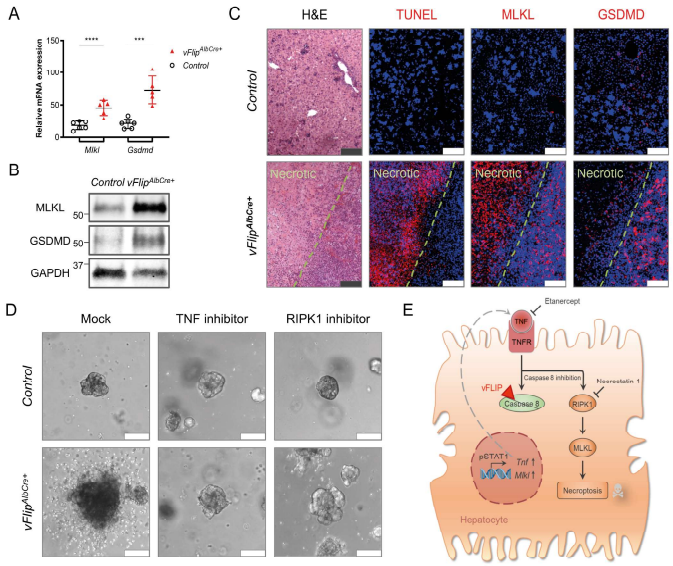
To evaluate the contribution of MLKL to hepatocellular necrosis-induced liver dysfunction we developed a new mouse model that is characterized by acute, cell death mediated liver failure. To this end, we took advantage of the fact that many viruses produce proteins actively interfering with the host-cell death machinery in order to prevent the elimination of the infected cell. In this process, caspase-8 represents a decisive 'checkpoint' in anti-viral response due to its function as apoptosis initiator. Therefore, several viruses like the human herpesvirus 8 (HHV8) produce proteins directly inhibiting caspase-8 activation. To elucidate the impact of these viral cell death manipulating proteins on liver homeostasis in vivo, we generated mice constitutively expressing vFLIP in hepatocytes. Surprisingly, we uncovered that these mice died within hours post birth. Histopathological analysis revealed that transgenic mice exhibited severe liver injury with typical characteristics of acu-



Transgenic vFLIP induces hepatocellular death
(A) Mice expressing vFLIP in hepatocytes. (B) Survival analysis starting from birth. (C) Liver morphology (80h post birth). (D) Serum AST and ALP levels. (E) H&E, and TUNEL (red) and vFLIPGFP-IRES (green) stained liver sections.

PD Dr. Dr. Günther

PD Dr. Dr. Wirtz



vFLIP expression triggers regulated necrosis
(A) qPCR analysis of liver Mlkl and Gsdmd expression. (B) Western Blot analysis (C) Staining of neonatal liver sections. (D) Images of treated liver organoids. (E) Schematic overview of vFLIP induced regulated necrosis in the liver.

te viral hepatitis and acute-on-chronic liver failure including yellowish discoloration of the liver, elevated serum amino transaminase levels and extended areas of hepatocellular necrosis. On a molecular level, we identified that effector proteins of regulated necrosis, in particular, necroptosis (MLKL) and pyroptosis (GSDMD) were elevated on gene expression and protein level. Interestingly, immunohistochemical stainings revealed a specific localization pattern of these effector proteins dependent on the proximity to the necrotic tissue suggesting a central role of necroptosis in hepatocellular death. Functional analysis using the liver organoid system clarified that vFlip expressing hepatocytes die via a RIPK1-MLKL mediated necroptosis driven by an autocrine TNF production. These findings not only highlight the potential of viral cell death regulatory molecules in manipulating the host-cell death response in vivo, but also the crucial role of different forms of regulated necrosis in the context of hepatic diseases.

PD Dr. Dr. Claudia Günther
phone: +49 9131 85 45210
e-mail: c.guenther@uk-erlangen.de

Publications during funding period

Gunther C, Ruder B, Stolzer I, Dorner H, He GW, Chiriac MT, Aden K, Strigli A, Bittel M, Zeissig S, Rosenstiel P, Atreya R, Neurath MF, Wirtz S, Becker C (2019) Interferon Lambda Promotes Paneth Cell Death Via STAT1 Signaling in Mice and Is Increased in Inflamed Ileal Tissues of Patients With Crohn's Disease. Gastroenterology. 157(5):1310-22.e13

Bittel M, Kremer AE, Sturzl M, Wirtz S, Stolzer I, Neurath MF, Ballon G, Gunther C (2019) Modulation of the extrinsic cell death signaling pathway by viral Flip induces acute-death mediated liver failure. Cell Death & Disease. 10(12):878

Stolzer I, Kaden-Volynets V, Ruder B, Letizia M, Bittel M, Rausch P, Basic M, Bleich A, Baines JF, Neurath MF, Wirtz S, Weidinger C, Bischoff SC, Becker C, Günther C (2019) Environmental Microbial Factors Determine the Pattern of Inflammatory Lesions in a Murine Model of Crohn's Disease–Like Inflammation. Inflammatory Bowel Diseases 26(1):66-79

Ruder B, Murtadak V, Stürzl M, Wirtz S, Distler U, Tenzer S, Mahapatro M, Greten FR, Hu Y, Neurath MF, Cesarman E, Ballon G, Günther C, Becker C (2018) Chronic intestinal inflammation in mice expressing viral Flip in epithelial cells. Mucosal Immunology.

Hefele M, Stolzer I, Ruder B, He GW, Mahapatro M, Wirtz S, Neurath MF, Gunther C (2018) Intestinal epithelial Caspase-8 signaling is essential to prevent necroptosis during Salmonella Typhimurium induced enteritis. Mucosal Immunology. 11(4):1191-202

He GW, Gunther C, Kremer AE, Thonn V, Amann K, Poremba C, Neurath MF, Wirtz S, Becker C (2017) PGAM5-mediated programmed necrosis of hepatocytes drives acute liver injury. Gut. 66(4):716-23

Gunther C, He GW, Kremer AE, Murphy JM, Petrie EJ, Amann K, Vandenabeele P, Linkermann A, Poremba C, Schleicher U, Dewitz C, Krautwald S, Neurath MF, Becker C, Wirtz S (2016) The pseudokinase MLKL mediates programmed hepatocellular necrosis independently of RIPK3 during hepatitis. The Journal of Clinical Investigation. 126(11):4346-60

Newly started Projects

A76 01/02/2020 - 31/07/2022

Role of Gasdermin C in Gut Barrier Defence


Prof. Dr. Becker

Prof. Dr. Christoph Becker,
Department of Medicine 1 – Gastroenterology, Pneumology and Endocrinology

We have discovered Gasdermin C as a protein strongly induced in the gut epithelium by IL-4 and IL-13. Our preliminary data further suggest that Gasdermin C can be released from epithelial cells and may bind to bacterial surfaces. Further analyses implicate that Gasdermin C has a pore forming function and promotes anti-microbial defence. We plan to elucidate the regulation of Gasdermin C, its molecular mode of action and its functional impact in vivo.

e-mail: christoph.becker@uk-erlangen.de

A77 30 months

HIF expression in B cells regulates bone loss


Prof. Dr. Bozec

Prof. Dr. Aline Bozec, Department of Medicine 3 – Rheumatology and Immunology

While the influence of T cells on bone homeostasis has been well characterized, less is known about the role of B cells. Despite that B cells are able to produce RANKL, the major cytokine regulating osteoclast differentiation, its regulation of expression remains unclear. B cells reside in the low oxygen concentrations bone niche, and adapt to the environment through the expression of HIFs. I therefore hypothesize that HIF expression in B cells could influence the development of osteoporosis.

e-mail: aline.bozec@uk-erlangen.de

A78 30 months

Smurf2-IFN axis in IBD and mucosal healing


Dr. Dr. Chiriac


Prof. Dr. Neurath

Dr. Dr. Mirea Chiriac, Prof. Dr. Markus Neurath
Department of Medicine 1 – Gastroenterology, Pneumology and Endocrinology

To understand the role played by ubiquitination of type I interferon in the pathogenesis of inflammatory bowel disease we intend to induce DSS colitis in two newly generated conditional mouse strains i.e. Stat2 and Smurf2 in experimental colitis models. CRISPR/Cas, three dimensional organoids coupled with Nanostring and RNA-Seq/GO analysis will be used to understand molecular mechanisms underlying DSS findings. Data will be validated using samples from IBD patients and controls.

e-mail: mirea.chiriac@uk-erlangen.de

A79 30 months

TR4 in tissue fibrosis


Prof. Dr. Distler

Prof. Dr. Jörg Distler, Department of Medicine 3 – Rheumatology and Immunology

Fibrotic diseases account for 45% of the deaths in the developed world. We demonstrate that the nuclear receptor TR4 is overexpressed in fibrotic tissues in a TGFbeta-dependent manner. TR4 promotes fibroblast-to-myofibroblast transition and collagen release. Knockout of TR4 prevents fibroblast activation and ameliorates experimental fibrosis. In the proposed project, we aim to characterize the molecular mechanisms of fibroblast activation by TR4 and the antifibrotic effects of TR4 inhibition.

e-mail: joerg.distler@uk-erlangen.de

Newly started Projects

A80 01/01/2020 - 30/06/2022

Inflammasomes in primary dendritic cells

Prof. Dr. Dudziak

Prof. Dr. Diana Dudziak, Department of Dermatology

Inflammasomes play a pivotal role in the immune response against pathogens, but also in the pathogenesis of inflammatory disorders. Our data indicate that inflammasome activation in DCs is leading to full DC stimulation without induction of pyroptosis. We hypothesize that uncontrolled inflammasome stimulation in DCs might be key component in inflammatory disorders. In this study, we want to elucidate the mechanisms in this specific inflammasome activation in primary DC.

e-mail: diana.dudziak@uk-erlangen.de

A81 01/01/2020 - 30/06/2022

Receptor and neuropathogenicity of Bornavirus

Prof. Dr. Ensser

Prof. Dr. Armin Ensser, Institute of Clinical and Molecular Virology

Recently, we detected Borna disease virus (BoDV-1) as the cause of human fatal encephalitis. Previous studies have addressed the immune response and viral replication, but the host cell receptor of BoDV-1 remained unknown. We will use an unique BoDV-1 patient isolate to search for this receptor, and we will address the possible direct, non-immune related neuropathogenic potential of BoDV-1, as well as antiviral (chemo)therapeutic options, in IPSC derived human neuronal 3D organoid cultures.

e-mail: armin.ensser@uk-erlangen.de

A82 01/02/2020 - 31/07/2022

Role of RANTES in the resolution of asthma

Prof. Dr. Finotto

Prof. Dr. Susetta Finotto, Department of Medicine 1 – Gastroenterology, Pneumology and Endocrinology

We identified RANTES as a key regulator of the resolution of allergic asthma in human and murine studies. Resolved symptomatic episodes of asthma in children, were found to be associated with elevated serum levels of RANTES indicating the involvement of RANTES in the resolution of allergic asthma. In a murine model after allergen (HDM) challenge, RANTES cured allergic asthma trait. In this project, we want to better understand the mechanism of RANTES mediated resolution of allergic asthma.

e-mail: susetta.neurath-finotto@uk-erlangen.de

A83 01/01/2020 - 30/06/2022

The role of SAMHD1 in CMV/ HIV coinfections

Prof. Dr. Gramberg

Prof. Dr. Thomas Gramberg, Institute of Clinical and Molecular Virology

HIV patients coinfected with CMV show increased morbidity and mortality, even on therapy. Despite high coinfection rates, surprisingly little is known about molecular interactions of CMV and HIV. We found that CMV blocks the HIV restriction factor SAMHD1 to facilitate its own replication. This finding finally provides a handle to explain how CMV enhances HIV replication in the host. Thus, we will address the working hypothesis that CMV infection boosts HIV replication by inactivating the SAMHD1.

e-mail: thomas.gramberg@uk-erlangen.de

Newly started Projects

A84 01/06/2020 - 30/11/2022

Tissue-resident memory T cells in GvHD


Prof. Dr. Hildner


Prof. Dr. Büttner-Herold


Dr. Zundler

Prof. Dr. Kai Hildner, Dr. Sebastian Zundler,
Department of Medicine 1 – Gastroenterology, Pneumology and Endocrinology
Prof. Dr. Maike Büttner-Herold,
Department of Nephropathology

T cell mediated intestinal inflammation in acute Graft-versus-Host-Disease (GI-GvHD) represents a life-threatening and therapeutically challenging complication in patients undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT). Interestingly, the role of tissue-resident memory T cells (Trm) in this context is unknown. Here, we plan studies to assess the development, migration, location and functionality of Trm cells in GI-GvHD both in murine experimental models and in men.

e-mail: kai.hildner@uk-erlangen.de

A85 30 months

The pathophysiology of SAPHO syndrome


Prof. Dr. Hüffmeier

Prof. Dr. Ulrike Hüffmeier, Institute of Human Genetics

SAPHO syndrome is a rare inflammatory disease of the skeleton and skin with unsolved etiology, but suspected causal/ disease-contributing genetic factor(s). We identified several rare PLXNA1 variants in patients with SAPHO syndrome. We propose to identify the molecular mechanisms by those human variants that lead to disease. Our study will allow to understand the etiology of SAPHO and to pave the way for planned analyses in vertebrates and genetic follow-up studies.

e-mail: ulrike.hueffmeier@uk-erlangen.de

A86 30 months

Charakterization of synovial macrophage subsets

Prof. Dr. Gerhard Krönke, Department of Medicine 3 – Rheumatology and Immunology

Prof. Dr. Krönke

Our preliminary data identified a unique Cx3Cr1-positive macrophage subset that forms a protective barrier around the joint and counteracts inflammation. Accordingly, we will address the developmental origin and differentiation pathways of these specific macrophages and try to understand the molecular basis of their anti-inflammatory properties. Moreover, we will address the relevance of these findings for human diseases such as rheumatoid arthritis.

e-mail: gerhard.kroenke@uk-erlangen.de

A87 30 months

DC subsets and natural antibodies in leishmaniasis

Dr. Christian Lehmann, Department of Dermatology,
PD Dr. Ulrike Schleicher, Institute of Microbiology – Clinical Microbiology, Immunology and Hygiene

Dr. Lehmann

PD Dr. Schleicher

Dendritic Cells (DCs) are indispensable for the protection from pathogens. Additionally, natural antibodies (nAbs) reacting to evolutionary conserved epitopes foster fast targeted response. Leishmaniasis is an important tropical disease with different manifestations. However, the first events in infection and determination of T/NK cell responses by DCs and nAbs are not fully understood. We now aim to unravel early determining factors for clinical outcome in leishmaniasis on a single cell level.

e-mail: christian.lehmann@uk-erlangen.de

Newly started Projects

A88 01/02/2020 - 30/11/2022

Cyclin interaction with a CDK-like viral kinase


Prof. Dr. Marschall


Prof. Dr. Sticht

Prof. Dr. Manfred Marschall, Instiute of Clinical and Molecular Virology
Prof. Dr. Heinrich Sticht, Institute of Biochemistry

HCMV replication is characterized by viral CDK-cyclin interaction. The CDK-like viral kinase pUL97 interacts with human cyclins. CycB1 is phosphorylated upon the interaction, dependent on pUL97 activity, whereas cycT1/H interaction stimulates pUL97 activity and substrate phosphorylation. Regions for cyclin interaction and antiviral drug resistance show overlaps in pUL97, so that this correlation will be elucidated in terms of viral fitness for the development of a novel antiviral strategy.

e-mail: manfred.marschall@fau.de

A89 30 months

CD83 regulates homeostasis and inflammation


Prof. Dr. Steinkasserer

Prof. Dr. Alexander Steinkasserer, Department of Immune Modulation

Inflammation within the CNS can directly affect neuronal structures. Thus, molecules controlling inflammatory responses are of upmost importance. The immune-regulatory CD83 molecule is highly expressed by microglia and tissue-resident macrophages and thus, represents a crucial factor for microglial activation and the neuro-immune crosstalk. Since, its regulation and function in these cells has not been elucidated we will investigate this during immune homeostasis and neuroinflammation.

e-mail: alexander.steinkasserer@uk-erlangen.de

A90 01/01/2020 - 30/06/2022

The fate of lung-resident memory T-cells

Prof. Dr. Tenbusch

Prof. Dr. Matthias Tenbusch, Institute of Clinical and Molecular Virology

In this project, we will analyse the regulatory mechanism for the maintenance of lung-resident memory T-cells. The impact of secondary events of inflammation or infections on the pre-existing immunity will be the major focus. Furthermore, we determine whether the differential induction of the immunity by either a primary infection or a gene-based vaccine might play a role in this context.

e-mail: matthias.tenbusch@fau.de

Newly started Projects

A91 16/03/2020 - 15/09/2022

Interfering with HTLV-1 persistence

Dr. Thoma-Kreß

Dr. Andrea Thoma-Kreß, Institute of Clinical and Molecular Virology

The highly oncogenic retrovirus Human T-cell leukemia virus type 1 (HTLV-1) causes incurable neoplastic or inflammatory diseases. The viral accessory protein p8, which is proteolytically cleaved from the pre-cursor p12 and transported to target cells prior to infection, is important for establishing persistent infections in vivo. Here, we aim to identify the protease cleaving p12 into p8, to inhibit this protease, and to assess the impact of blocking of p12/p8 processing on viral persistence.

e-mail: andrea.thoma-kress@uk-erlangen.de

A92 01/02/2020 - 30/11/2022

FRCs and immune tolerance induction

Prof. Dr. Mario Zaiss

Prof. Dr. Mario Zaiss, Department of Medicine 3 – Rheumatology and Immunology

As lymphatics in the inflamed joint in rheumatoid Arthritis drain specifically the popliteal lymph node (pLN) where the adaptive immune response is initiated, we investigated a population of stromal cells in the pLN, namely the fibroblastic reticular cells (FRC). Our preliminary data show a significant immunomodulatory potential of pLN FRCs in inflammatory arthritis mouse models. Therefore, we hypothesize that specifically pLN stromal FRCs play a so far neglected role in the early onset of RA.

e-mail: mario.zaiss@uk-erlangen.de

D24 - Progress Report

01/06/2016 - 31/05/2019

Differentiation-associated Schwann cell transcription factors in melanoma - learning from embryogenesis

Prof. Dr. Anja Bosserhoff, Prof. Dr. Michael Wegner, Institute of Biochemistry

Melanoma is an aggressively disseminating cancer with continuously rising incidence. Melanoma cells derive from melanocytes, which originate from the neural crest and display characteristics of cells of the nervous system. Interestingly, Schwann cells, nervous system cells derived from the neural crest, can transdifferentiate into melanocytes and vice versa. Based on the expertise of both PIs, the project is on central Schwann cell transcription factors and their role in melanoma.

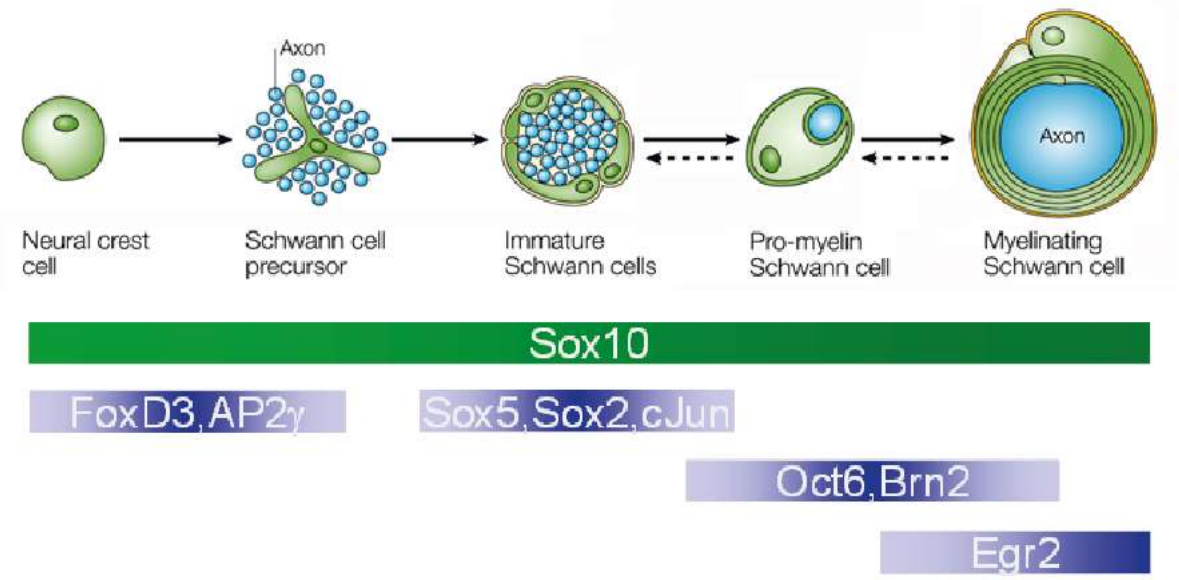
Aims in this project:

1. Definition of differentiation-associated Schwann cell transcription factors that play a role in melanoma

2. Determination of molecular differences and similarities between schwannomas and melanomas

At the beginning of the project we started to determine, which of the transcription factors that are important in Schwann cells during development and in the adult differentiated cells, are deregulated in melanoma development or progression. We were able to define several Schwann cell transcription factors as strongly deregulated in melanoma cell lines and in tissue material compared to melanocytes including TFAP2C and EGR2.

In subsequent studies, we have focused on the transcription factor EGR2, which is most strongly deregulated, and analysed its specific impact on melanoma in detail. EGR2 is one of the key drivers of myelination in Schwann cells, where it is induced in a Sox10-dependent manner early during terminal differentiation and then regulates (in cooperation with Sox10) target genes that code for structural proteins of the myelin sheaths or for proteins involved in lipid metabolism. However, regarding melanoma few data are available so far. Expression and activity of EGR2 in melanoma was confirmed using various analyses including EMSA. On a functional level, upon EGR2 downregulation via siRNA, melanoma cells re-



Transcription factors involved in differentiation of Schwann cells.

Prof. Dr. Bosserhoff Prof. Dr. Wegner

duced their rate of proliferation and their migratory potential. In clonogenic assays, the number and size of colonies was significantly altered upon knockdown of EGR2. These findings point towards a possible role of EGR2 in melanoma progression. To get an initial idea whether EGR2 influences lipid metabolism in melanoma cells as it does in Schwann cells, we analyzed the triglyceride content in melanoma cells. Upon treatment with siRNA against EGR2, the triglyceride amount was substantially reduced in three independent melanoma cell lines supporting the assumption that EGR2 is at least in part responsible for the high triglyceride levels in melanoma. As one important target gene of EGR2 in melanoma we determined DGAT2. Functional assays revealed that targeting DGAT2 by siRNA resulted in similar effects as targeting EGR2. Now, we are following up on the idea of an impact on myelination of these factors. We are planning to generate melanoma and Schwann cell lines in which EGR2 is deleted by CRISPR/Cas9. The resulting transcription factor-deficient cell lines will be compared in their expression profile to the original ones and between melanoma and Schwann cell line.

In summary, we use knowledge from Schwann cell differentiation to define central transcriptional regulators for melanoma development and progression, which have not been associated with pathogenesis before, and thereby obtain a better molecular and cellular understanding of this tumour entity.

Schwann cells also give rise to tumours. The resulting schwannomas are mostly benign, slow growing and thus very different from melanoma. In a second part of the project we therefore compare the mRNA expression pattern of schwannomas and melanomas to set the basis for a characterisation of genes that promote or repress the metastatic process in melanoma.

In summary, we use knowledge from Schwann cell differentiation to define central transcriptional regulators for melanoma development and progression, which have not been associated with pathogenesis before, and thereby obtain a better molecular and cellular understanding of this tumour entity.

Contact:

Prof. Dr. Bosserhoff
phone: +49 9131 85 24190
e-mail: anja.bosserhoff@fau.de

Prof. Dr. Wegner
phone: +49 9131 85 24620
e-mail: michael.wegner@fau.de

Publications during funding period

Truch K, Arter J, Turnescu T, Weider M, Hartwig AC, Tamm ER, Sock E, Wegner M (2018) Analysis of the human SOX10 mutation Q377X in mice and its implications for genotype-phenotype correlation in SOX10-related human disease. Hum. Mol. Genet. 27: 1078–1092

Dietrich P, Kuphal S, Spruss T, Hellerbrand C,Bosserhoff AK (2018) Wild-type KRAS is a novel therapeutic target for melanoma contributing to primary and acquired resistance to BRAF inhibition. Oncogene 37: 897–911

Stieglitz D, Lamm S, Braig S, Feuerer L, Kuphal S, Dietrich P, Arndt S, Echtenacher B, Hellerbrand C, Karrer S, Bosserhoff AK (2019) BMP6-induced modulation of the tumor micro-milieu. Oncogene 38: 609–621

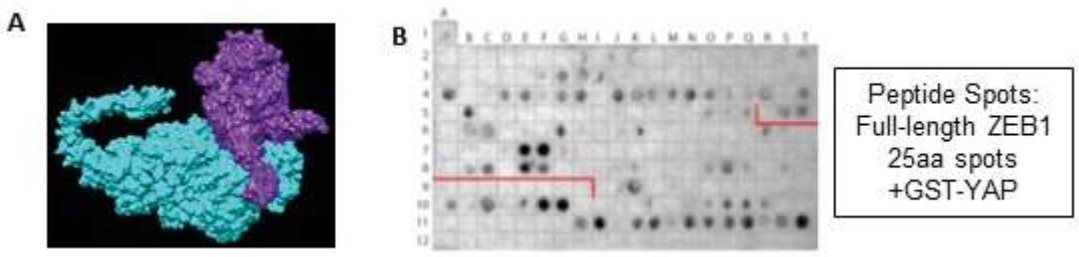
D25 - Final Report

01/05/2016 - 30/04/2019

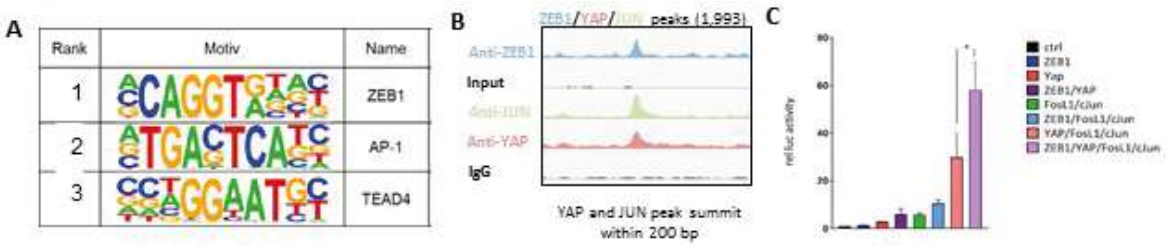
Interaction of the EGFR- and the ZEB1-pathway in aggressive cancer types

Prof. Dr. Thomas Brabletz, Chair of Experimental Medicine I – Molecular Pathogenesis Research

The EMT-program provides cancer cells with motility, invasiveness and stem cell features. A major EMT inducer is the transcriptional ZEB1. However, many of the underlying molecular mechanisms of its tumor promoting effects are unknown. To clarify the versatile functions of ZEB1, we validated, verifid and mapped interactions with novel interaction partners identified by MassSpec and ChIP-Seq analyses. We further investigated their relevance for Zeb1 function and cancer progression.



(A) In silico medelling of the 3D interaction between ZEB1 (green) and YAP1 (lilac) (B) Validation of the proposed interaction domains and further characterization the interactions motifs by peptide spots.



(A) Zeb1 ChIP-Seq peaks enrich for AP-1 (Jun/Fos) and Tead/Yap motives. (B) Strongly overlapping peaks after ChIP-Seqs for Zeb1, Jun and Yap1 on the promoter of a putative, tumor-promoting target genes. (C) Functional validation of the interaction of all three factors on the target gene promoter using reporter assays.

Prof. Dr. Brabletz

We could show that under certain conditions - e.g. in an oncogenic context of cancer cells – the oncogenic factor ZEB1 can switch from a transcriptional repressor of epithelial genes to a transcriptional activator of tumor promoting genes. We proposed novel nuclear interaction partners and previously exemplified this by showing interaction with YAP1, a main effector of Hippo signalling, to activate a specific common target gene set. In order to identify additional coactivators of ZEB1, we had performed Co-IPs from nuclear extracts of aggressive cancer cells, coupled to mass-spec and proteomic analyses and had detected about 20 unknown nuclear co-factors of ZEB1. Among the top 5 identified putative co-factors of ZEB1 were the nuclear EGFR and STAT3, AP-1 factors and YAP1. In the first year of funding, we confirm the nuclear interactions of EGFR and Stat 3 with Zeb1 by applying CoIPs and proximity ligation assays in tumor cells and IL6-activated fibroblasts. In parallel we started analyses assessing a functional cooperation to activate a common target genes set. In addition we further mapped the detected interaction of ZEB1 with the Hippo-Pathway effector YAP1, e.g. by applying an in silico modelling approach (in cooperation with Dr. Viji Mahadevan, Bangalore, India). Their functional interaction was also confirmed by detection of the TEAD-motif (TEAD=YAP1 DNA-binding partner) as one of the top3 DNA binding motifs in a ZEB1 ChIP-Seq.Furthermore a second unbiased strategy was applied to identify functional cooperation partners of Zeb1 in promoting tumor progression. To this end we analysed Zeb1 ChIP-Seq-data sets (performed partially with support of the IZKF high tech pool) allowing the identification candidate interaction partners on tumor cell enhancers and promoters. Motif searches on Zeb1 ChIP Seq peaks revealed a strong overlap of with binding sites for the transcription factors ZEB1, AP-1 (Jun/Fos) and Tead (confirming the interaction with the Tead partner YAP1). Direct comparison of ChIP Seq peaks for Zeb1, Jun and Yap1 revealed a strong overlap in target enhancers and promoters. Their functional cooperation to commonly activate tumor promoting genes was further validated, e.g. in reporter assays.

Contact:
Prof. Dr. Brabletz
phone: +49 9131 85 29104
e-mail: thomas.brabletz@fau.de

Invited lectures

Cellular plasticity in cancer: driving force and therapeutic target, Febr. 4. 2019, 1. Deutscher Krebsforschungskongress, Heidelberg

EMT and stemness in cancer, 1. Deutscher Krebsforschungskongress, Febr. 4. 2019, Heidelberg

Krebsmetastasen – fataler Prozess, faszinierende Mechanismen, zukünftige Therapieansätze, Universitätsbund Erlangen-Nürnberg, April 8. 2019

Cellular plasticity in cancer: driving force and therapeutic target, Symposium FOR 2127 – Metastatic Cancer Progression, June 5. 2019, Regensburg

Publications during funding period

none

D27 - Final Report

01/07/2016 - 30/06/2019

2-Hydroxyglutarate in Acute Myeloid Leukaemia: Novel Molecular Targets and Impact on Immune Escape

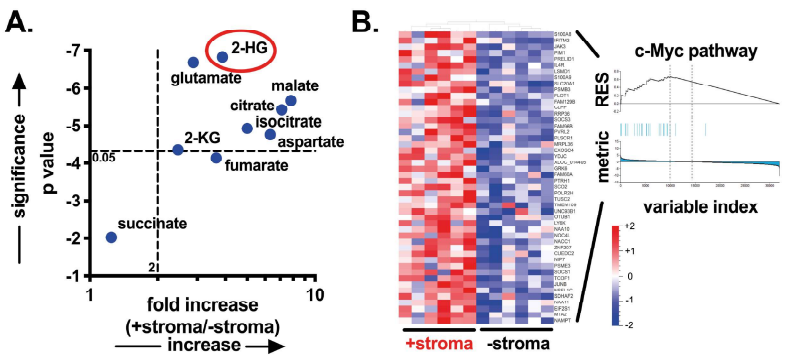
Prof. Dr. Dimitrios Mougiakakos, Department of Medicine 5 – Haematology and Oncology

Increased 2-hydroxyglutarate (2-HG) levels are found in 15% of acute myeloid leukaemia (AML) patients. 2-HG overproduction is attributed to mutations in isocitrate dehydrogenase 1/2 (IDH1/2). Our data indicates a link between increased 2-HG levels and c-Myc pathway. AML patients display substantial immune defects. Several tumor-derived metabolites hamper immune responses. The impact of 2-HG remains unexplored. Our aim is to investigate the impact of 2-HG on immune responses and to identify targetable pathways contributing to its production.

Our findings using mass spectrometry show an increased 2-HG production in patient-derived AML-cells carrying wild-type IDH1/2 after being cultured in presence of a human bone marrow-derived stromal cell line (HS-5). We could recapitulate those observations when testing AML cell lines (e.g. KG1, OCI-AML, and THP1) that are negative for IDH mutations in an equivalent manner. In fact, co-culturing AML-cells isolated from different patients with stromal cells led to marked alterations of their gene expression profile. Among the significantly upregulated genes (upon stromal contact) we found several candidates belonging to the c-Myc signaling pathway when performing gene set enrichment analyses. In line, c-Myc protein levels were also significantly increased.

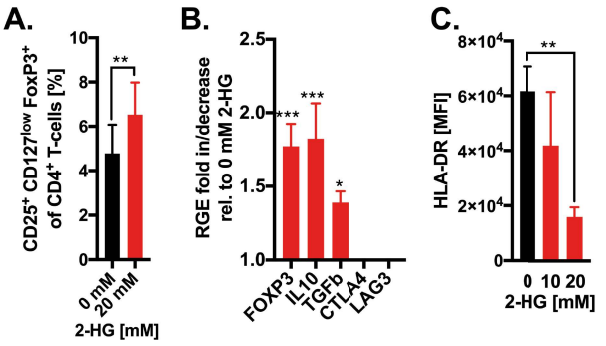
Similar to observations from other disease models 2-HG accumulation was associated with a HIF1-α stabilization under normoxic conditions and an increase of intracellular ROS levels, which (together with the c-Myc upregulation) could contribute as a so-called "pseudohypoxic response" to the marked glycolytic skewing. The role of c-Myc for such metabolic transition is further underscored by experiments revealing a reduced lactic acid production (indicative for aerobic glycolysis) when treating (c-Myc+) AML cell lines with the pharmacological c-Myc inhibitor 10058-F4 in non-cytotoxic concentrations. In fact, preliminary data suggest that the cells' c-Myc levels might correlate with the intracellular content of 2-HG in the tested AML cell lines and in analogy to previous observations from studies in breast cancer and multiple myeloma.

Accumulation of cancer-associated metabolites such as ROS or lactic acid has been previously linked to immune modulation. Therefore, we were interested whether 2-HG holds potential immune regulatory properties by interfering with T-cell function. We found that exogenously applied, non-cell-permeable 2-HG was readily taken up by T-cells. While viability, proliferation, and IFNγ production were mainly unaffected by 2-HG, bioenergetics of activated T-cells shifted away from aerobic glycolysis towards



AML-cells were cultured +/- stromal cells. (A) Metabolites were quantified by mass spectrometry (n=6). (B) Microarray analyses were performed and top upregulated genes in AML-cells in presence of stromal cells are shown as a heat map together with a GSEA enrichment plot for the c-Myc pathway.

Prof. Dr. Mougiakakos



(A, B) T-cells were activated +/- 2-HG. Induction of CD25+CD127lowFoxP3+ TRegs by 2-HG. Increased expression levels of genes linked to TReg differentiation and function. (C) Monocytes treated with 2-HG and assessment of HLA-DR reduction indicative for MDSC induction.

respiration. This is at least partly explained by the observed 2-HG-triggered hypoxia inducible factor-1α (HIF-1α) destabilization and despite the upregulation of genes linked to adaptation towards tissue hypoxia. In line with previous findings that HIF-1α-dependent (aerobic) glycolysis orchestrates differentiation of Th17 cells, we found a reduced Th17 polarization in presence of 2-HG together with lower levels of circulating Th17 cells in AML patients.

Contact:
Prof. Dr. Mougiakakos
phone: +49 9131 85 43172
e-mail: dimitrios.mougiakakos@uk-erlangen.de

Invited lectures

State-of-the Art lecture, 20.11.2019, Hof, “CAR T cell therapy today and tomorrow”

10. Oncological Symposium, 20.11.2019, Bayreuth, “Modern immunotherapies in lymphoma”

I-O Academy, 08.11.2019, Hamburg, “Tumor metabolism as barrier for the immune system”

DGHO, 14.10.2019, Berlin, “Metabolic Targeting in Immuno-Oncology”

9. KOK Meeting, 06.09.2019, Berlin, “CAR T cell therapy – challenges in daily practice”

5. Porto Cancer Meeting, 02.05.2019, Porto, “Metabolic interactions in CLL”

Post-ASCO 2019, 06.07.2019, Nürnberg, “Hematological malignancies“

Meet the Expert, 08.05.2019, Nürnberg, “Sequential therapy in CLL”

Educational lecture, 28.03.2019, Bamberg, “Therapy landscape in CLL”

Post-ASH 2018, 30.01.2019, Erlangen, „Aggressive lymphomas”

Awards

Gilead oncology research award, 12.10.2019, Berlin

Publications during funding period

Böttcher M, Renner K, Berger R, Mentz K, Thomas S, Cadenas EZ, Dettmer K, Oefner PJ, Mackensen A, Kreutz M, Mougiakakos D (2018) D-2-hydroxyglutarate interferes with HIF-1a stability skewing T-cell metabo-lism towards oxidative phosphorylation and impairing Th17 polarization. OncoImmunology, 7(7):e1445454

D28 - Final Report

01/02/2016 - 31/01/2019

SPARCL1 function in vessel maturation and metastasis of colorectal carcinoma

Prof. Dr. Michael Stürzl, PD Dr. Elisabeth Naschberger, Department of Surgery

In previous work we demonstrated tumor microenvironment (TME)-dependent heterogeneity of tumor endothelial cells (TECs) in colorectal carcinoma (CRC) and identified SPARCL1 as an important regulatory molecule of TME-associated vessel homeostasis and vascular-derived inhibition of tumor growth. Here we investigated the functions and underlying mechanisms of SPARCL1 in physiological angiogenesis.

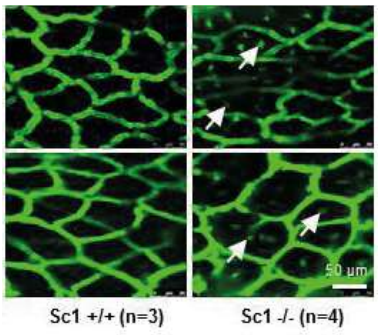
The specific aims of the project were:

Aim 1: Structure-function analyses of SPARCL1 and isolation of its cellular receptor

Wild type recombinant human SPARCL1 (hSPARCL1) has been successfully cloned and was purified from supernatants of human eukaryotic cells. The protein showed similar anti-angiogenic activity compared to commercially recombinant hSPARCL1. Subdomains of hSPARCL1 were cloned and purified and are presently used for structure-function analysis of the anti-angiogenic activity of hSPARCL1. CD105/endoglin was identified as a cellular receptor of hSPARCL1 in HeLa cells.

Aim 2: Mapping of SPARCL1 expression between different species

For subsequent mechanistic studies in mice, SPARCL1 expression was systematically analyzed in different human and mouse tissues, both, by western blot and at the single cell level by immunofluorescence analyses. Murine SPARCL1 (mSPARCL1) was most strongly expressed in the lung, colon and stomach, intermediately in esophagus, coecum, heart, spleen, urinary bladder and absent in the liver, kidney and small intestine. Immunofluorescence analyses in humane tissues showed coherently with the results obtained in mice a strong expression of hSPARCL1 in in stomach, large intestine and lung. Interestingly, SPARCL1 expression in mouse tissues was mostly associated with mural cells whereas in human tissues it was expressed in both, mural and endothelial cells. These analyses revealed that SPARLC1 expression is strongly associated with vascular cell types and exhibits organ specific variations.



SPARCL1 knockout mice exhibit increased colon vessel permeability. FITC-dextran was injected into SPARCL1 wt (Sc1+/+) and KO (Sc1-/-) mice. Accumulation of FITC-dextran in the crypts as a marker of increased permeability was recorded.

Aim 3: Impact of SPARCL1 in physiological angiogenesis

In order to analyze the impact of SPARCL1 on angiogenesis/vessel maturation in vivo the metatarsal angiogenesis assay (cooperation Ramming/Wohlfahrt, Med3) has been established. Metatarsal bones from embryos of wild type SPARCL1 animals were explanted at E18.5 and cultivated under conditions allowing outgrowth of endothelial sprouts with a supporting feeder layer. An in vivo inhibition of vessel sprouting by addition of recombinant mSPARCL1 was detected. Moreover, metatarsals from SPARCL1-ko mice showed altered vessel morphology with dilated and fused vessels characteristically associated with reduced vessel maturation and increased permeability. In accordance with this, an increased vessel permeability to FITC-dextran was detected in the colon of SPARLC1 ko mice. These results suggested that SPARCL1 regulates physiological vessel function.

Aim 4: Impact of SPARCL1 on prognosis and therapy response of patients with CRC

Human SPARCL1 expression was determined in RNA extracted from FFPE-tissue sections of CRC patient samples (n=614, Polyprobe study). Implementati-


Prof. Dr. Stürzl


PD Dr. Naschberger

on of the clinical data in adequate software tools (TranSMART, cooperation Christoph/Prokosch, MIK) to analyze potential clinical correlations was conducted. A reduced incidence of metastases in the long-term follow up of R0-resected CRC patients with high SPARCL1 expression was identified.

Contact:
PD Dr. Naschberger
phone: +49 9131 85 39524
e-mail: elisabeth.naschberger@uk-erlangen.de

Invited lectures

Stürzl M. Pathogenic Impact of the Interplay of Inflammation and Blood Vessels in Colorectal Carcinoma and Inflammatory Bowel Diseases, DKFZ-BioMedX-Lunch Talk Series, Nov. 21. 2019, Heidelberg

Publications during funding period

Langer V, Vivi E, Regensburger D, Winkler TH, Waldner MJ, Rath T, Schmid B, Skottke L, Lee S, Jeon NL, Wohlfahrt T, Kramer V, Tripal P, Schumann M, Kersting S, Handtrack C, Geppert CI, Suchowski K, Adams RH, Becker C, Ramming A, Naschberger E, Britzen-Laurent N, Stürzl M. (2019) IFN-γ drives inflammatory bowel disease pathogenesis through VE-cadherin-directed vascular barrier disruption. J Clin Invest. 129(11):4691-4707. doi: 10.1172/JCI124884

Wohlfahrt T, Rauber S, Uebe S, Luber M, Soare A, Ekici A, Weber S, Matei AE, Chen CW, Maier C, Karouzakis E, Kiener HP, Pachera E, Dees C, Beyer C, Daniel C, Gelse K, Kremer AE, Naschberger E, Stürzl M, Butter F, Sticherling M, Finotto S, Kreuter A, Kaplan MH, Jüngel A, Gay S, Nutt SL, Boykin DW, Poon GMK, Distler O, Schett G, Distler JHW, Ramming A (2019) PU.1 controls fibroblast polarization and tissue fibrosis. Nature. doi: 10.1038/s41586-019-0896-x

Bardenbacher M, Ruder B, Britzen-Laurent N, Schmid B, Waldner M, Naschberger E, Scharl M, Müller W, Günther C, Becker C, Stürzl M*, Tripal P (2019) Permeability analyses and three-dimensional imaging of interferon gamma-induced barrier disintegration in intestinal organoids. Stem Cell Research. (in press) *corresponding author

Ruder B, Murtadak V, Stürzl M, Wirtz S, Distler U, Tenzer S, Mahapatro M, Greten FR, Hu Y, Neurath MF, Cesarman E, Ballon G, Günther C, Becker C (2018) Chronic intestinal inflammation in mice expressing viral Flip in epithelial cells. Mucosal Immunol. 2018 Nov;11(6):1621-1629

Ellmann S, Langer V, Britzen-Laurent N, Hildner K, Huber C, Tripal P, Seyler L, Waldner M, Uder M, Stürzl M, Bäuerle T (2018) Application of machine learning algorithms for multiparametric MRI-based evaluation of murine colitis. PLoS One. 13(10):e0206576

Unterer B, Wiesmann V, Gunasekaran M, Sticht H, Tenkerian C, Behrens J, Leone M, Engel FB, Britzen-Laurent N, Naschberger E, Wittenberg T, Stürzl M (2018) IFN-γ-response mediator GBP-1 represses human cell proliferation by inhibiting the Hippo signaling transcription factor TEAD. Biochem J. 475(18):2955-2967

Naschberger E, Regensburger D, Tenkerian C, Langheinrich M, Engel FB, Geppert C, Hartmann A, Grützmann R, Schellerer VS, Stürzl M (2018) Isolation of human endothelial cells from normal colon and colorectal carcinoma - an improved protocol. JoVE, (134)

Naschberger E, Geißdörfer W, Bogdan C, Tripal P, Kremmer E, Stürzl M, Britzen-Laurent N (2017) Processing and secretion of guanylate binding protein-1 depend on inflammatory caspase activity. J Cell Mol Med. 21(9):1954-1966

Gründner J, Prokosch HU, Stürzl M, Croner R, Christoph J, Toddenroth D (2018) Predicting Clinical Outcomes in Colorectal Cancer Using Machine Learning. Stud Health Technol Inform. 247:101-105

López Posadas R, Stürzl M, Atreya I, Neurath MF, Britzen-Laurent N (2017) Interplay of GTPases and cytoskeleton in cellular barrier defects during gut inflammation. Front. Immunol. 8.1240

He G-W, Günther C, Thonn V, Yu Y-Q, Martini E, Buchen B, Neurath M, Stürzl M, Becker C (2017) Regression of apoptosis-resistant colorectal tumors by induction of necroptosis in mice. The Journal of Experimental Medicine 214, 1655-1662

Bonsignore L, Passelli K, Pelzer C, Perroud M, Konrad A, Thurau M, Stürzl M, Dai L, Trillo-Tinoco J, Del Valle L, Qin Z, Thome M (2017) A role for MALT1 activity in Kaposi's sarcoma-associated herpesvirus latency and growth of primary effusion lymphoma. Leukemia 31, 614-624

Naschberger E, Liebl A, Schellerer VS, Schütz M, Britzen-Laurent N, Kölbel P, Schaal U, Haep L, Regensburger D, Wittmann T, Klein-Hitpass L, Rau TT, Dietel B, Méniel VS, Clarke AR, Merkel S, Croner RS, Hohenberger W, Stürzl M (2016) Matricellular protein SPARCL1 regulates tumor microenvironment-dependent endothelial cell heterogeneity in colorectal carcinoma. J Clin Invest. 126(11):4187-4204*
*highlighted in: Thomas, H.: CRC endothelial regulation. Nature Reviews Gastroenterology & Hepatology (2016) doi:10.1038/nrgastro.2016.180

Britzen-Laurent N, Herrmann C, Naschberger E, Croner RS, Stürzl M (2016) Pathophysiological role of guanylate-binding proteins in gastrointestinal diseases. World J Gastroenterol 22(28):6434-43

Croner RS, Sevim M, Metodiev MV, Jo P, Ghadimi M, Schellerer V, Brunner M, Geppert C, Rau T, Stürzl M, Naschberger E, Matzel KE, Hohenberger W, Lottspeich F, Kellermann J (2016) Identification of Predictive Markers for Response to Neoadjuvant Chemoradiation in Rectal Carcinomas by Proteomic Isotope Coded Protein Label (ICPL) Analysis. Int J Mol Sci 17(2).pii: E209

Feiersinger F, Nolte E, Wach S, Rau TT, Vassos N, Geppert C, Konrad A, Merkel S, Taubert H, Stürzl M, Croner RS (2016) MiRNA-21 expression decreases from primary tumors to liver metastases in colorectal carcinoma. PLoS One 11, e0148580

Newly started Projects

D30 30 months

Functional role of axin anchoring to microtubules in Wnt signaling


Prof. Dr. Behrens


Dr. Bernkopf

Prof. Dr. Jürgen Behrens, Dr. Dominic Bernkopf,
Chair of Experimental II – Molecular Oncology

Axin is a key negative regulator of the oncogenic Wnt/beta-catenin pathway scaffolding the beta-catenin destruction complex. We suggest that the newly found anchoring of axin to microtubules (MTs) is of functional importance for regulating the pathway. We will (i) describe the dynamics of axin association with MTs; (ii) determine the biochemical basis of this interaction and its regulation by phosphorylation; and (iii) define the functional role of axin anchoring to MTs in Wnt signaling.

e-mail: juergen.behrens@fau.de

D31 16/03/2020 - 15/09/2022

Modulation of oncogene-induced senescence by cell-matrix adhesion


Prof. Dr. Bosserhoff

Prof. Dr. Anja Bosserhoff, Institute of Biochemistry

Oncogene-induced senescence (OIS) was recently introduced as a strong tumor suppressive mechanism seen e.g. in development of nevi out of melanocytes after BRAF mutation. Tumor cells like melanoma obviously can overcome these limiting mechanisms by further changes, however the molecular mechanisms leading to and overcoming OIS are just being started to be understood. We aim to understand the role of cell adhesion processes and mechanotransduction in induction and overcoming OIS.

e-mail: anja.bosserhoff@fau.de

D32 01/03/2020 - 31/08/2022

NPY in chemo-resistance and immune-escape in HCC


Dr. Dietrich

Dr. Peter Dietrich,
Department of Medicine 1 – Gastroenterology, Pneumology and Endocrinology

Neuropeptide Y (NPY) and its receptors represent a highly conserved system which is involved in cancer-related hallmarks. However, the impact of the NPY-system on hepatocellular carcinoma (HCC) remains unclear. The aims of this study are i) to unravel the role of NPY-receptor/NPY-crosstalk in resistance to tyrosine kinase inhibitors such as sorafenib and lenvatinib in HCC, and ii) to analyze the unknown role of the NPY-system as a potential major determinant of immune-escape in HCC.

e-mail: peter.dietrich@uk-erlangen.de

D33 01/07/2020 - 31/12/2022

Immunometabolism in CML


Prof. Dr. Metzler


Prof. Dr. Mougiakakos

Prof. Dr. Markus Metzler,
Department of Pediatric and Adolescent Medicine
Prof. Dr. Dimitrios Mougiakakos,
Department of Medicine 5 – Haematology and Oncology

Despite the improvement through tyrosine kinase inhibitors (TKIs), treatment resistance, relapse and therapy-induced side effects are central problems of CML therapy. Our interdisciplinary project addresses the question whether and how TKIs alter CML cell metabolism and induce synthetic lethality in combination with compounds specifically targeting metabolic pathways. Our approach could help to improve efficacy and reduce side effects of CML treatment in pediatric and adult patients alike.

e-mail: markus.metzler@uk-erlangen.de

Newly started Projects

D34 30 months

Role of fibroblast polarization in the pathogenesis of colorectal carcinoma


PD Dr. Ramming


Prof. Dr. Stürzl

PD Dr. Andreas Ramming, Department of Medicine 3 - Rheumatology and Immunology
Prof. Dr. Michael Stürzl, Department of Surgery

We have identified PU.1 as a key regulator of fibroblast polarization. Its role in colorectal carcinoma (CRC) is unknown. We will address the following aims: (1) characterization of cancer-associated fibroblast (CAF) heterogeneity in CRC, (2) analysis of CAF polarization-dependent fibrocrine effects in vitro and (3) in experimental animal models, and (4) validation of the results in CRC tissues. Deciphering the role of fibroblast polarization in CRC may provide a new target for therapy.

e-mail: andreas.ramming@uk-erlangen.de

D35 30 months

Interactions of DPF3 and hypoxia in renal cancer


PD Dr. Schödel

PD Dr. Johannes Schödel,
Department of Medicine 4 - Nephropathology and Hypertension

The renal cancer risk SNP on chromosome 14q24.2 creates a novel Hypoxia inducible transcription factor (HIF)-binding DNA element in an intronic region of the DPF3 gene, a member of the SWI/SNF chromatin remodelling complex. DPF3 is upregulated in a SNP- and HIF-dependent fashion in renal tubular cells. We investigate the regulation of DPF3 in renal cells and cancer as well as its contribution to global chromatin status and transcription factor binding to critical DNA regions.

e-mail: johannes.schoedel@uk-erlangen.de

D36 01/03/2020 - 31/08/2022

Endogenous retroviruses drive tumor inflammation

Prof. Dr. Strick Prof. Dr. Hartmann

Prof. Dr. Reiner Strick, Department of Obstetrics and Gynaecology
Prof. Dr. Arndt Hartmann, Institute of Pathology

This proposal will focus on the molecular basis of tumor inflammation of two different advanced cancers; Bladder cancer (MIBC) and Ovarian cancer (OVCA) with poor survival outcomes and high recurrence rates. Endogenous retrovirus (ERV) activations are linked with innate immunity and tumor inflammation. We will correlate patient immune signatures with ERVs and determine the functional role of ERVs, including dsRNA and RNA/DNA intermediates using tumors, cell lines and tumoroids of MIBC and OVCA.

e-mail: reiner.strick@uk-erlangen.de

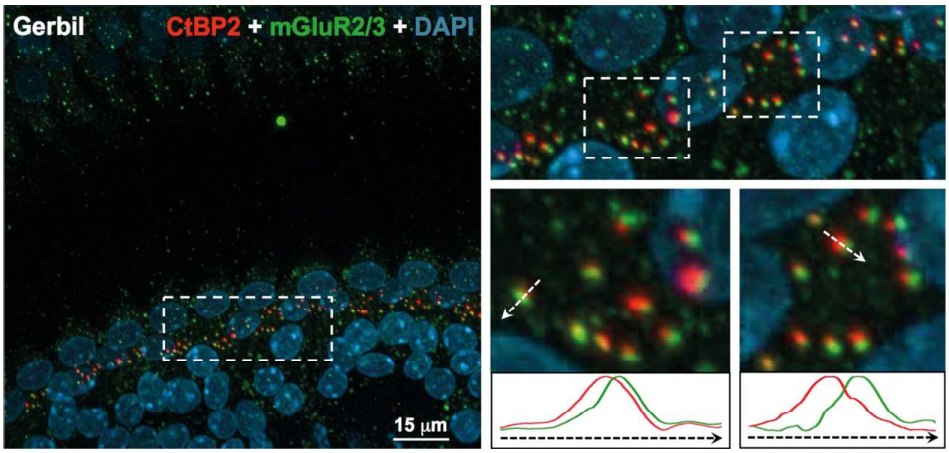
E19 - Final Report

15/02/2016 - 14/02/2019

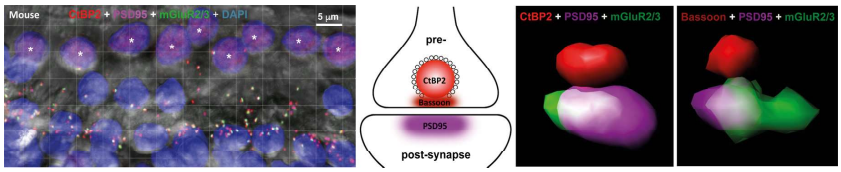
Inhibitory neurotransmission in the cochlea: Glutamate and endocannabinoids

Prof. Dr. Ralf Enz, Institute of Biochemistry

Glutamate and endocannabinoid receptors can regulate activity and survival of sensory neurons via inhibitory feedback loops. While inhibitory circuits of photoreceptors in the retina are well described, corresponding protective mechanisms in hair cells of the cochlea are largely unknown. Since sensory organs need a tailor-made regulation of their signal transduction pathways, this project investigates receptor expression in hair cells and elucidates their regulation by interacting proteins.



Staining of cochlear wholemounts for mGluR2 and mGluR3 (green) showed puncta in close vicinity to presynaptic ribbons (red). This is best seen in the magnification of the boxed regions. Fluorescence profiles were measured along the arrows and signal intensities are compared in the graphs.



(left) Cochlear wholemounts were stained for mGluR2/3, pre- and post-synaptic markers of inner hair cell synapses, as indicated in the sketch. (right) 3D-reconstructions show a clear post-synaptic localisation of the receptors. Inner hair cell nuclei are marked with asterisks.

Prof. Dr. Enz

Introduction

Neurotransmitter receptors represent major determinants that control neuronal signal transduction at synapses. There, the receptors interact with multiple proteins, such as enzymes and scaffolds that regulate their trafficking, localization, ligand affinity, desensitization behaviour and surface concentration. In this way, receptors and regulatory proteins assemble into synaptic signal complexes.

Receptor guided inhibitory feedback loops are important factors for activity and survival of sensory neurons, as well as for protection against noxious stimuli. G-protein coupled metabotropic glutamate receptors (mGluRs) expressed at pre- or post-synaptic sites can invert the activity of the excitatory neurotransmitter glutamate into neuronal inhibition and thus are well suited to build inhibitory feedback loops in glutamatergic neurons. The same holds true for pre-synaptically localized endocannabinoid (CB) receptors.

While molecular mechanisms of synaptic inhibition were analysed in detail in the retina, the identity of inhibitory protective circuits in the cochlea is not well understood. Based on our previous findings, we hypothesize that different sensory organs, e.g. the retina and the cochlea, need a tailor-made regulation of their synaptic signal complexes. In this project, we therefore analyse receptors and regulatory binding partners in hair cells of the cochlea.

Which inhibitory mGluR and CB receptors are expressed in the cochlea?

In contrast to mGluR1 and mGluR5, expression and localization of mGluR2, mGluR3, mGluR4, mGluR7a, mGluR7b, mGluR8a, mGluR8b and CB receptors in the inner ear is largely unknown. Here, we detected transcripts for all receptor types in the mouse cochlea. Furthermore, cochlear wholemounts of gerbil or mouse incubated with antibodies specific for mGluR2/3, mGluR4, mGluR8 or CB2 showed punctate signals, indicating synaptic localization of these receptors. Signals for mGluR2/3 were present at ribbon synapses of inner hair cells. Using combinations of confocal microscopy, super resolution STED microscopy and 3D reconstructions, we found a co-localization of mGluR2/3 with post-synaptic (PSD95), but not with pre-synaptic (CTBP2, Bassoon) markers at inner hair cell ribbon synapses.

How are cochlear mGluR2/3 regulated?

Given the post-synaptic expression of mGluR2/3 at inner hair cell ribbon synapses, we searched for intracellular proteins that bind to and thereby regulate receptor function. Yeast 2-hybrid screens using intracellular C-termini of mGluR2 or mGluR3 as baits for a cochlear cDNA-library yielded several hundred potential interaction partners that were clustered in functional groups, representing proteins involved in post-translational modifications, trafficking, cell adhesion or of the cytoskeleton. Of these, pull-down assays showed robust and reproducible interaction of 3 proteins with the receptors´ C-termini. The molecular and functional characterization of these protein-protein interactions is on-going.

Contact:
Prof. Dr. Enz
phone: +49 9131 85 24185
e-mail: ralf.enz@fau.de

Publications during funding period

Klotz L, Wendler O, Frischknecht R, Shigemoto R, Schulze H, Enz R (2019) Localization of group II and III metabotropic glutamate receptors at pre- and postsynaptic sites of inner hair cell ribbon synapses. FASEB J 33:13734-13746 doi: 10.1096/fj.201901543R

E20 - Final Report

01/05/2016 - 30/04/2019

Identification of molecules, receptors and genes involved in chronic pruritus

PD Dr. Dr. Andreas Kremer, Department of Medicine 1 - Gastroenterology, Pneumology and Endocrinology
Prof. Dr. Katharina Zimmermann, Department of Anesthesiology

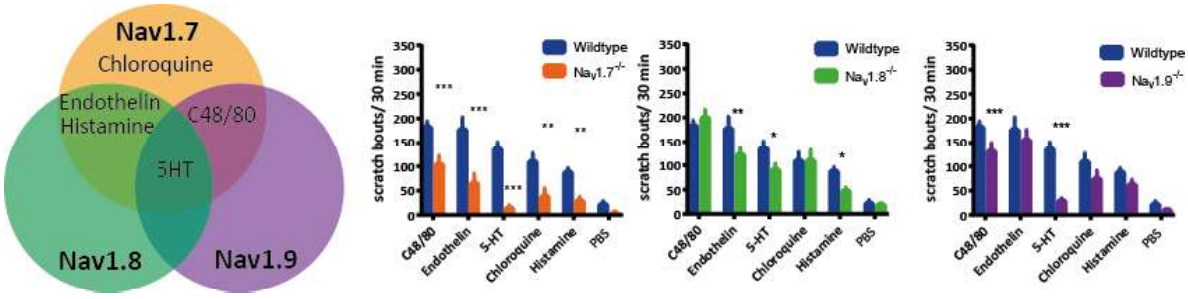
Chronic pruritus is a distressing symptom accompanying many dermatological and systemic disorders. The three aims of this project are to (I) identify pruritogens in plasma of patients suffering from chronic pruritus, to (II) characterize the specific voltage-gated sodium channel (NaV) subtypes that generate and propagate the action potentials in itch pathways, and, (III) to identify and characterize novel gene products that predispose or protect from itch by quantifying phenotypic differences in scratch behavior in inbred mouse strains.

Identification of pruritogens in plasma of patients suffering from chronic pruritus

Several members of the Mas-gene related receptor (MRG) family are selectively activated by pruritogens leading to activation of primary sensory neurons. To elucidate a potential contribution to itch signaling in chronic pruritus in patients, we established two screening methodologies to screen patient serum and pharmaceutical drugs for MRG receptor activation. Thereby, we identified novel agonists of MRGX2 which lead to mast cell degranulation and potentially itch.

Identification of specific NaV channel subtypes required for itch signaling

Three NaV channel subtypes, NaV 1.7, NaV1.8 and NaV 1.9, are restricted to the peripheral sensory system and play essential roles in the generation and transmission of action potentials. They have been suggested as potential drug targets for itch. Since the precise contribution of these three different subtypes in itch signaling of different acute stimuli remains elusive, we used murine knockout models and assessed the scratch behavior upon intradermal injection of 10 different pruritogens. DRGs isolated from knockout and wildtype mice showed an equal depolarization capacity upon stimulation with different pruritogens measured by calcium imaging and exhibited only minor neurophysiological differences. Scratching behavior after intradermal injection of the pruritogens in the nuchal fold differed in the three knockout mice as compared to wildtype mice. Noticeably, the deletion of single NaV channels led to highly variable deficits in scratching behavior suggesting an unexpectedly high complexity of itch signaling. Nonetheless, NaV1.7 was essential for all strong pruritogens and seemingly functions as the threshold channel and is a key channel for the effects of all potent pruritogens. NaV 1.9 and NaV1.8 probably function as amplifiers for some pruritogens or as high-threshold backup for long-lasting depolarizations, respectively.



Intradermal injection of pruritogens caused variably reduced scratching in NaV1.7-/-,1.8-/- and 1.9-/- mice showing a diversity in NaV-dependent itch signalling (N=10-12). The results also are summarized in a Venn-diagram.


PD Dr. Dr. Kremer


Prof. Dr. Zimmermann

Identification of novel genes that modulate pruritus severity in mice

Individual and ethnical differences in the experience of pruritus are recognized challenges in the treatment of pruritus. To investigate heritable differences in the sensitivity to pruritogens we used an automated scratch assay and evaluated scratch behavior in 20 inbred mouse strains subsequent to intradermal injection of 10 commonly known pruritogens. We found a large influence of the genetic background with numerous strains being highly sensitive and other strains with resistance to some or all pruritogens. The trait values served to perform haplotype-based computational genetic mapping (HBCGM) and led to the identification of genes with single-nucleotide polymorphisms and high correlation with the trait value variability. Subsequent pathway analysis, literature and expression database searches led to the selection of 35 candidate genes. Some of them will be subject of further molecular biology, electrophysiology and behavioral studies.

Contact:
PD Dr. Dr. Kremer
phone: +49 9131 85 45015
e-mail: andreas.kremer@uk-erlangen.de

Prof. Dr. Zimmermann
phone: +49 9131 85 33901
e-mail: katharina.zimmermann@fau.de

Invited lectures

DGVS Seminar Hepatologie, 06.03.19, Berlin, „Primär biliäre Cholangitis und immun-vermittlete Cholangiopathien“

International Liver Meeting, 06.04.19, Wien, “Histological outcomes with long-term obeticholic acid therapy“

Grand Round, 23.04.19, Universität Innsbruck, “Cholestase und Pruritus“

Pharmacon, Bundesapothekertagung, 27.05.19, Meran, “ Chronischer Pruritus – Ursachen und Therapie“

China Lecture Tour, 06/19, Xi’an, Nanjing, Foshan, u.a., „NASH: the new epidemic in hepatology”

DGVS Tagung, 03.10.19, Wiesbaden, “PBC: Obeticholsäure versus Bezafibrat“

Publications during funding period

Dhillon AK, Kremer AE, Kummen M, Boberg KM, Elferink RPO, Karlsen TH, Beuers U, Vesterhus M, Hov JR (2019) Autotaxin activity predicts transplant-free survival in primary sclerosing cholangitis. Sci Rep 9(1):8450

Robering JW*, Gebhardt L*, Wolf K, Kühn H, Kremer AE*, Fischer MJM* (2019) Lysophosphatidic acid activates satellite glia cells and Schwann cells. Glia 67(5):999-1012 *contributed equally

Schmid R*, Wolf K*, Robering JW, Strauß S, Strissel PL, Strick R, Rübner M, Fasching PA, Horch RE, Kremer AE, Boos AM, Weigand A (2018) ADSCs and adipocytes are the main producers in the autotaxin-lysophosphatidic acid axis of breast cancer and healthy mammary tissue in vitro. BMC Cancer 18(1):1273.

Kremer AE, Le Cleac’h A, Lemoinne S, Wolf K, De Chaisemartin L, Chollet-Martin S, Humbert L, Rainteau D, Poupon R, Rousseau A, Chazouillères O, Corpechot C (2019) Antipruritic effect of bezafibrate and serum autotaxin measures in patients with primary biliary cholangitis. Gut. 68(10):1902-1903

He GW, Günther C, Kremer AE, Thonn V, Amann K, Poremba C, Neurath MF, Wirtz S, Becker C (2017) PGAM5-mediated programmed necrosis of hepatocytes drives acute liver injury. Gut 66(4): 716-723

Günther C, He GW, Kremer AE, Murphy JM, Petrie EJ, Amann K, Vandenabeele P, Linkermann A, Poremba C, Schleicher U, Dewitz C, Krautwald S, Neurath MF, Becker C, Wirtz S (2016) The pseudokinase MLKL mediates programmed hepatocellular necrosis independently of RIPK3 during hepatitis. J Clin Invest. 126(11): 4346-4360

Wunsch E, Krawczyk M, Milkiewicz M, Trottier J, Barbier O, Neurath MF, Lammert F, Kremer AE*, Milkiewicz P* (2016) Serum Autotaxin is a Marker of the Severity of Liver Injury and Overall Survival in Patients with Cholestatic Liver Diseases. Sci Rep. 6: 30847 *contributed equally

Kühn H, Kappes L, Wolf K, Gebhardt L, Neurath MF, Reeh P, Fischer MJM, Kremer AE (2020) Complementary roles of murine NaV1.7, NaV1.8 and NaV1.9 in acute itch signaling. Sci Rep. 10: 2326

E21 - Final Report

01/05/2016 - 30/04/2019

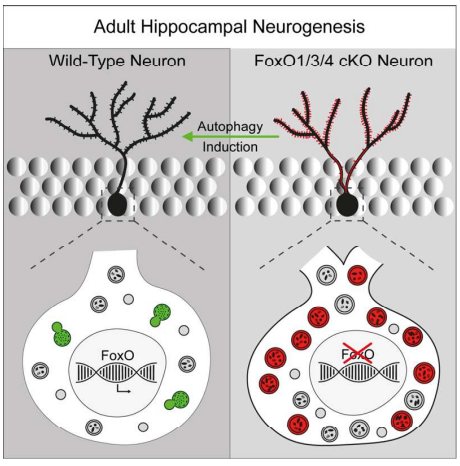
Modulation of alpha-Synuclein pathology by FoxO-dependent pathways

Prof. Dr. Dieter Chichung Lie, Institute of Biochemistry
Prof. Dr. Jochen Klucken, Department of Molecular Neurology

Dysregulation of autophagy, the central cellular self-clearance mechanism, is impaired in synucleinopathies including Parkinson´s disease and has been implicated in the cell-to-cell transfer of aSyn potentially leading to disease progression. This project has adressed the currently unresolved question of how ageing accelerates aSyn-related toxicity and cerebral spreading, and how autophagy interplays with the ageing-associated FoxO-pathway.

FoxO transcription factors potently modulate autophagy in neural cells

Autophagy is a highly conserved catabolic pathway with emerging functions in human neurodevelopmental and neurodegenerative diseases. The FoxO transcription factors are not only considered important regulators of the ageing process but have recently been identified as modulators of the course of inflammatory and degenerative diseases in humans. In this project we identified FoxOs as major transcriptional regulators of autophagic flux in the adult hippocampus. Specifically, we demonstrated that the generation of new neurons from stem cells in the adult hippocampus is dependent on FoxO activity and showed that conditional deletion of the Forkhead Box O transcription factors FoxO1, FoxO3, and FoxO4 strongly impaired autophagic flux in newly generated neurons. Impaired autophagic flux was associated with excessive altered synaptic integration and survival of neurons. Strikingly, pharmacological induction of autophagy was sufficient to correct abnormal dendrite and spine development of FoxO-deficient neurons. Collectively, these findings uncover a novel link between FoxO transcription factors, autophagic flux, and maturation of developing neurons and provide the basis for future studies that will analyze whether FoxO-dependent autophagy is involved in the pathophysiology of neurodevelopmental and neurodegenerative diseases.



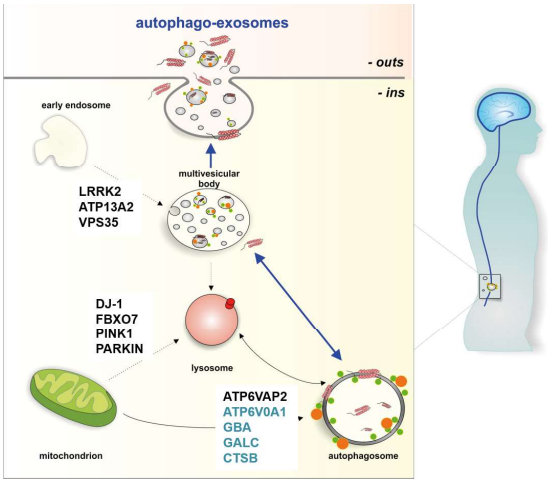
FoxO transcription factors regulate the activity of the autophagolysosomal pathway in newly generated neurons (left). Loss of FoxOs (right) impairs autophagic flux, leading to excessive synapse formation and aberrant integration of neurons (modified from Schäffner et al., 2018)

Autophagy induction by running as a protective factor in synucleinopathies

The second aspect of the project addressed the interplay of cellular autophagy with systemic changes related to aging. In this phase of the project we analyzed the characteristics of a unique and novel feature of extracellular vesicles – “exosomes” released by neural cells after autophagy inhibition which we termed “autophagoexosomes”. They could be identified in the human CSF of affected patients and supported the spreading of synucleinopathy in rodent brains. Cerebral autophagy was inducted by a physical exercise model in in alpha-synuclein transgenic mice leading to a change in phenotype (gait and postural control patterns) and neuropathology patterns. 4 weeks treadmill exercise intervention in adult human alpha-synuclein expressing mice revealed that at baseline, alpha-synuclein mouse models exhibited irregular and less active gait, with impaired dynamic postural control, compared to wild type mice. Exercise particularly improved speed and stride length, while increasing dual diagonal versus three-paw


Prof. Dr. Lie


Prof. Dr. Klucken



Autophagoexosomes are released by neurons as a consequence of a disturbed autophagosomal processing of alpha-Synuclein and altered mitochondrial function. Several genes associated with aging and Parkinson's disease are involved in these cellular pathomechanisms.

body support in both the alpha-synuclein knockout and transgenic mice. Biochemical analyses showed higher striatal tyrosine hydroxylase immuno-reactivity and reduced higherorder alpha-synuclein species – a specific characteristic of the disease associated protein - in the cerebral cortex. This was an important finding since physical exercise was able to induce systemic autophagy and protect from the neurodegenerative phenotype, however, without affecting cerebral autophagy.

Contact:
Prof. Dr. Lie
phone: +49 9131 85 24622
e-mail: chi.lie@fau.de

Prof. Dr. Klucken
phone: +49 9131 85 34127
e-mail: jochen.klucken@uk-erlangen.de

Publications during funding period

Hoffmann, Minakaki, Menges, Salvi, Savitskiy, Kazman, Vicente Miranda, Mielenz, Klucken, Winkler and Xiang (2019) Extracellular aggregated alpha synuclein primarily triggers lysosomal dysfunction in neural cells prevented by trehalose Sci Rep, 9: 544

Timotius, Canneva, Minakaki, Moceri, Plank, Casadei, Riess, Winkler, Klucken, Eskofier and von Horsten (2019) Systematic Data Analysis and Data Mining in CatWalk Gait Analysis by Heat Mapping Exemplified in Rodent Models for Neurodegenerative Diseases J Neurosci Methods: 108367

Minakaki, Canneva, Chevessier, Bode, Menges, Timotius, Kalinichenko, Meixner, Muller, Eskofier, Casadei, Riess, Schroder, Winkler, Xiang, von Horsten and Klucken (2019) Treadmill exercise intervention improves gait and postural control in alpha-synuclein mouse models without inducing cerebral autophagy Behav Brain Res, 363: 199-215

Schaffner I, Minakaki G, Khan M A, Balta E A, Schlotzer-Schrehardt U, Schwarz T J, Beckervordersandforth R, Winner B, Webb A E, DePinho R A, Paik J, Wurst W, Klucken J & Lie D C (2018) FoxO Function Is Essential for Maintenance of Autophagic Flux and Neuronal Morphogenesis in Adult Neurogenesis. Neuron, 99, 1188-1203

Balta E A, Schaffner I, Wittmann M T, Sock E, von Zweydorf F, von Wittgenstein J, Steib K, Heim B, Kremmer E, Haberle B M, Ueffing M, Lie D C* & Gloeckner C J* (2018) Phosphorylation of the neurogenic transcription factor SOX11 on serine 133 modulates neuronal morphogenesis. Scientific Reports, 8 (1):16196 (*corresponding authors)

Balta E A, Wittmann M T, Jung M, Sock E, Haeberle B M, Heim B, von Zweydorf F, Heppt J, von Wittgenstein J, Gloeckner C J* & Lie D C* (2018) Phosphorylation Modulates the Subcellular Localization of SOX11. Frontiers in Molecular Neuroscience, 11:211. (*corresponding authors)

Jung M, Haberle B M, Tschaikowsky T, Wittmann M T, Balta E A, Stadler V C, Zweier C, Dorfler A, Gloeckner C J & Lie D C* (2018) Analysis of the expression pattern of the schizophrenia-risk and intellectual disability gene TCF4 in the developing and adult brain suggests a role in development and plasticity of cortical and hippocampal neurons. Molecular Autism, 9:20

Kavyanifar A, Turan S & Lie D C (2018) SoxC transcription factors: multifunctional regulators of neurodevelopment. Cell and Tissue Research, 371, 91-103

Minakaki G, Menges S, Kittel A, Emmanouilidou E, Schaeffner I, Barkovits K, Bergmann A, Rockenstein E, Adame A, Marxreiter F, Mollenhauer B, Galasko D, Buzás EI, Schlötzer-Schrehardt U, Marcus K, Xiang W, Lie DC, Vekrellis K, Masliah E, Winkler J, Klucken J. (2018) "Autophagy inhibition promotes alpha-synuclein release and transfer via extracellular vesicles with a hybrid autophago/exosome-like phenotype". Autophagy 14(1):98-119

Menges S, Minakaki G, Schaefer I, Meixner H, Prots I, Schlotzer-Schrehardt, Friedland K, Winner B, Outeiro T, Winklhofer J, von Arnim C, Xiang W, Winkler J and J Klucken (2017) Alpha-synuclein prevents the formation of spherical mitochondria and apoptosis under oxidative stress Sci Rep, 7: 42942

Beckervordersandforth R, Ebert B, Schaffner I, Moss J, Fiebig C, Shin J, Moore D L, Ghosh L, Trinchero M F, Stockburger C, Friedland K, Steib K, von Wittgenstein J, Keiner S, Redecker C, Holter S M, Xiang W, Wurst W, Jagasia R, Schinder A F, Ming G L, Toni N, Jessberger S, Song H and Lie D C (2017) Role of Mitochondrial Metabolism in the Control of Early Lineage Progression and Aging Phenotypes in Adult Hippocampal Neurogenesis. Neuron 93(6): 1518

E22 - Final Report

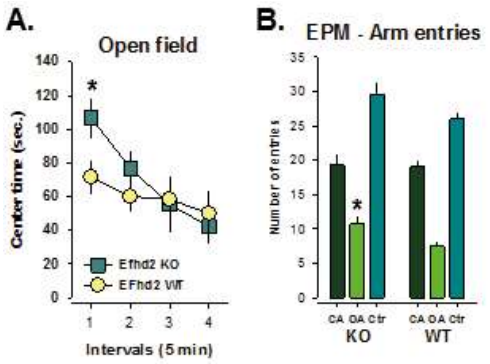
01/03/2016 - 28/02/2019

The role of Swiprosin-1/EFhd2 in resilience to drug addiction

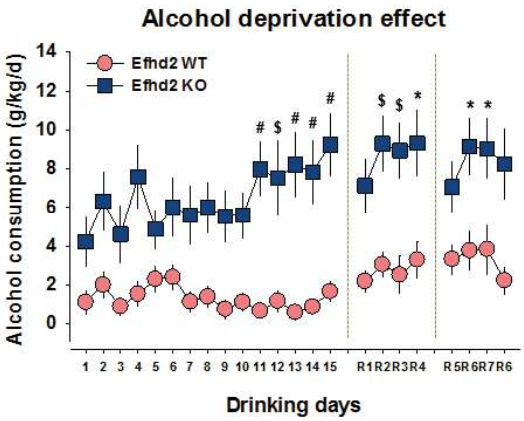
Prof. Dr. Christian Müller, Department of Psychiatry and Psychotherapy
Prof. Dr. Christian Alzheimer, Institute of Physiology and Pathophysiology
Prof. Dr. Dirk Mielenz, Department of Molecular Immunology

Drug addiction is a prevalent psychiatric disorder which develops from controlled consumption of psychoactive drugs. Normal behavioural traits, such as sensation seeking and/or low anxiety render an organism more or less susceptible to the addictive effects of alcohol. Present findings suggest that Swiprosin-1/ EFhd2 may be a resilience factor against the establishment of alcohol-, cocaine- and methamphetamine addiction.

In many societies, the majority of adults regularly consume alcohol. However, only a small proportion develops alcohol addiction. Individuals at risk often show a high sensation-seeking/ low anxiety behavioural phenotype. Here we asked which role EFhd2 (Swiprosin-1) plays in the control of alcohol addiction-associated behaviours. EFhd2 knock out (KO) mice drink more alcohol than controls and spontaneously escalate their consumption. This coincided with a sensation-seeking and low anxiety phenotype. A reversal of the behavioural phenotype with β-carboline, an anxiogenic inverse benzodiazepine receptor agonist, normalized alcohol preference in EFhd2 KO mice, demonstrating an EFHd2-driven relationship between personality traits and alcohol preference. These findings were confirmed in a human sample where we observed a positive association of the EFHD2 SNP rs112146896 with lifetime drinking and a negative association with anxiety in healthy adolescents. The lack of EFhd2 reduced extracellular dopamine levels in the brain, but enhanced responses to alcohol. In confirmation, gene expression analysis revealed reduced tyrosine hydroxylase expression and the regulation of genes involved in cortex development, Eomes and Pax6, in EFhd2 KO cortices. These findings were corroborated in Xenopus tadpoles by EFhd2 knock-down. Magnetic resonance imaging (MRI) in mice showed that a lack of EFhd2 reduces cortical volume in adults. Moreover, human MRI confirmed the negative association between lifetime alcohol drinking and superior frontal gyrus volume. These findings showed that EFhd2 is a conserved resilience factor against alcohol consumption and its escalation, working through Pax6/Eomes. Reduced EFhd2 function induces high-risk personality traits of sensation seeking/ low anxiety associated with enhanced alcohol consumption which may be related to cortex function. In a parallel study we found that EFhd2 also controls the establishment of the conditioned rewarding effects of cocaine and methamphetamine, two psychostimulant type drugs. EFhd2 is also here required to control the drug-induced ac-



EFhd2 knock out mice display a sensation seeking/ low anxiety behavioural phenotype in (A) the open field test and (B) the elevated plus maze.



The lack of Swirprosin-1/ EFhd2 in mice leads to enhanced consumption of alcohol in a free-choice drinking paradigm and spontaneous escalation of consumption.

Prof. Dr. Müller Prof. Dr. Alzheimer Prof. Dr. Mielenz

tivation of monoaminergic signalling in the brain as a functional marker for the rewarding effects of the drugs. These findings support the view that EFhd2 may not only provide resilience for alcohol addiction, but for drug addiction in general.

Contact:
Prof. Dr. Müller
phone: +49 9131 85 36896
e-mail: christian.mueller@uk-erlangen.de

Invited lectures

Exeter Addictions Workshop, University of Exeter, UK. 07.02.2019, Mechanisms of drug instrumentalization.

FAPESP/Baylat Meeting, Universität Erlangen, 29.03.2019, From mice to man and back again – Translational models of psychiatric disorders and their comorbidities

25. Wissenschaftliches Symposium des Norddeutschen Suchtforschungsverbundes e.V., Hannover, 08.05.2019, Paradoxe antidepressive Wirkung von Alkohol und mögliche pathophysiologische Mechanismen.

IBNS Meeting, Cairns, 25.06.2019, Common genetic base for sensation seeking and alcohol abuse – from mice to man.

Polish Academy of Sciences, Institute of Pharmacology, Krakau, 26.07.2019. The role neutral sphingomyelinase in alcohol consumption,

Neurons in Action 2019, Nencki Institute Warsaw, 12.09.2019 , Paradox antidepressant effects of alcohol and mechanisms of alcohol instrumentalization

Awards

Forschungspreis: Prädiktive, präventive und personalisierte Medizin in Psychiatrie und Neurologie der Deutschen Gesellschaft für Psychiatrie, Psychotherapie und Neurologie (DGPPN), Christian P. Müller, 30.11.2019, Berlin

Publications during funding period

Kogias G, Zheng F, Kalinichenko LS, Kornhuber J, Alzheimer C, Mielenz D, Müller CP (2020) Swirprosin1/ EFhd2 is involved in the monoaminergic and locomotor responses of psychostimulant drugs. Journal of Neurochemistry, in press

König L, Kalinichenko L S, Huber S E, Voll A M, Bauder M, Kornhuber J, Hausch F, Müller C P, (2019) The selective FKBP51 inhibitor SAFit2 reduces alcohol consumption and reinstatement of conditioned alcohol effects in mice. Addiction Biology, e12758, e12758. doi: 10.1111/adb.12758

Kogias G, Kornhuber J, Reimer D, Mielenz D, Müller CP (2019) Swiprosin-1/ EFhd2: from immune regulator to personality and brain disorders. NeuroSignals, 27(S1):1-19

Müller CP, Chung C, Qin L, Liu C, Xu B, Gao H, Ruggeri B, Hieber S, Schneider J, Jia T, Tay N, Akira S, Satoh T, Banaschewski T, Bokde ALW, Bromberg U, Büchel C, Quinlan EB, Floor H, Frouin V, Garavan H, Gowland P, Heinz A, Ittermann B, Martinot JL, Paillère Martinot ML, Artiges E, Lemaitre H, Nees F, Orfanos DP, Paus T, Poustka L, Millenet S, Fröhner JH, Smolka MN, Walter H, Whelan R, Bakalkin G, Liu Y, Desrivières S, Elliott P, Eulenburg V, Levy D, Crews F, Schumann G (2019) TANK controls alcohol drinking and cortical emotion pathways. Cerebral Cortex, 29: 1736-1751

Hoffmann AC, Minakaki G, Menges S, Salvi R, Savitskiy S, Kazman A, Miranda HV, Mielenz D, Klucken J, Winkler J, Xiang W. (2019) Extracellular aggregated alpha synuclein primarily triggers lysosomal dysfunction in neural cells prevented by trehalose. Scientific Reports, 9(1):544

Regensburger M, Loskarn S, Reimer D, Brachs S, Chichung L D, Mielenz D, Winner B (2018). Swiprosin-1/EFhd2 – a positive regulator of adult hippocampal neurogenesis. Stem Cell Reports, 10(2): 347-355

Mielenz D, Reichel M, Jia T, Quinlan EB, Stöckl T, Mettang M, Zilske D, Kirmizi-Alsan E, Schönberger P, Praetner M, Huber SE, Amato D, Schwarz M, Purohit P, Brachs S, Spranger J, Hess A, Büttner C, Ekici AB, Perez-Branguli F, Winner B, Rauschenberger V, Banaschewski T, Bokde AL, Büchel C, Conrod PJ, Desrivières S, Flor H, Frouin V, Gallinat J, Garavan H, Gowland P, Heinz A, Martinot JL, Lemaitre H, Nees F, Paus T, Smolka MN; IMAGEN Consortium., Schambony A, Bäuerle T, Eulenburg V, Alzheimer C, Lourdusamy A, Schumann G, Müller CP (2018) EFhd2/Swiprosin-1 is a common genetic determinator for sensation-seeking/low anxiety and alcohol addiction. Molecular Psychiatry 23(5): 1303-1319

Kalinichenko LS, Gulbins E, Kornhuber J, Müller CP (2018) The role of sphingolipids in psychoactive drug use and addiction. Journal of Neural Transmission, 125: 651-672

Huber SE, Zoicas I, Reichel M, Mühle C, Büttner C, Ekici AB, Lenz B, Kornhuber J, Müller CP (2018) Prenatal testosterone-receptor activation determines adult alcohol and water drinking in a gender-specific way. Addiction Biology 23(3): 904-920

Müller CP (2018) Animal models of psychoactive drug use and addiction – present problems and future needs for translational approaches, Behavioural Brain Research, 352: 109-115

Lang SC, Harre U, Purohit P, Dietel K, Kienhöfer D, Hahn J, Baum W, Herrmann M, Schett G, Mielenz D (2017) Neurodegeneration Enhances the Development of Arthritis. Journal of Immunology 198:2394–2402

Huber SE, Lenz B, Kornhuber J, Müller CP (2017) Prenatal androgen-receptor activity has organizational morphological effects in mice, PLOS One 12(11): e0188752

E25 - Final Report

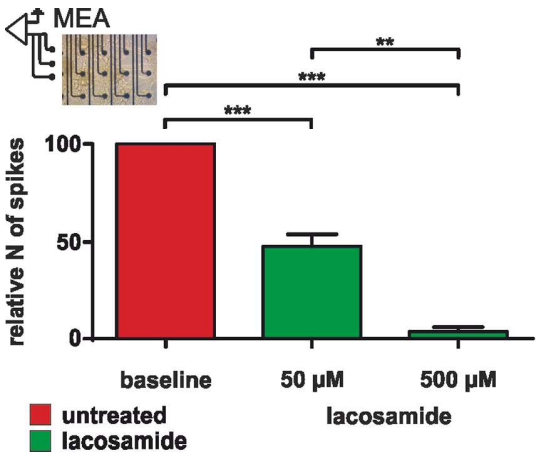
01/07/2016 - 30/06/2019

Modeling pain syndromes using human induced pluripotent stem cell-derived nociceptors

Prof. Dr. Beate Winner, Department of Stem Cell Biology
Prof. Dr. Dr. Jürgen Schüttler, Department of Anesthesiology

Our project investigated patient-derived sensory neurons from a patient affected by small fiber neuropathy that suffered from refractory neuropathic pain. Increased spontaneous activity of patient C-fibers was mirrored by hyperexcitability of patient-derived nociceptors and could be reverted by the FDA approved drug lacosamide. Based on these in-vitro findings we could predict an effective treatment in an individualized therapeutic approach.

Small fiber neuropathy (SFN) can manifest as chronic neuropathic pain syndrome characterized by severe burning pain in the extremities with limited therapeutic options. Although most SFNs are idiopathic, rare mutations in the sodium channels Nav1.7, Nav1.8 and Nav1.9 have been linked to SFN. We used a fibroblast reprogramming approach to generate human induced pluripotent stem cells (hiPSCs) and differentiate the hiPSC into nociceptors from a 69-year old Caucasian patient and age matched controls. The patient carried two rare variants in Nav1.9 (p.N1169S) and Nav1.8 (p.R923H) and suffered from SFN with refractory neuropathic pain for over ten years. All obtained hiPSC lines expressed the pluripotency markers NANOG and OCT3/4 and exhibited 92.6 – 99.0% TRA1-60-positive cells by FACS-analysis. When differentiated into stem cell-derived nociceptors all sensory neurons showed a comparable expression pattern of the peripheral neuron marker Peripherin and TUJ and also expressed the nociceptor specific markers TRPV1 and Nav1.9.



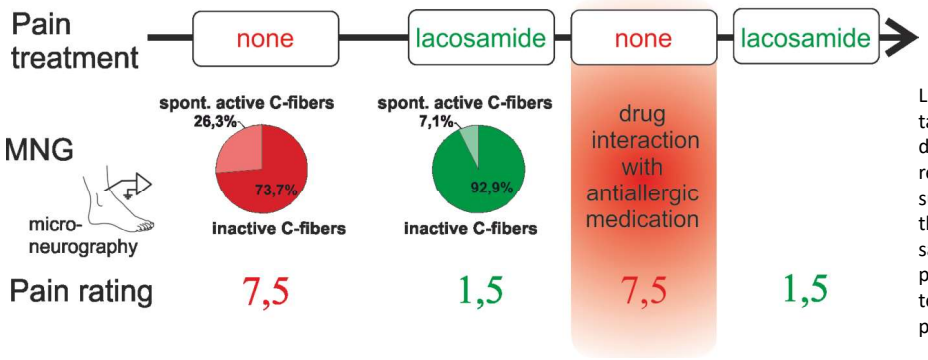
Experimental approach: Patient- and control-derived sensory neurons were generated from hiPSCs obtained by a fibroblast reprogramming approach and further characterised with molecular and electrophysiological methods.

Furthermore, we found that increased spontaneous activity of the patient's C-fibers investigated with microneurography recordings (26.3% compared to 11.8% in healthy age matched controls) was mirrored by in-vitro findings in patient-derived nociceptors. These showed increased spontaneous activity measured with patch-clamp technique in current-clamp mode (19.4% vs 1.8% in control sensory neurons) as well as an increased number of spikes when grown on multielectrode array (MEA) plates.

In MEA recordings, the FDA approved antiepileptic drug lacosamide (500 µM) strongly reduced the number of spikes in patient-derived nociceptors but not in control groups indicating that pathological hyperactivity is impaired but general action potential generation does not seem to be affected. Lacosamide was still effective on patient-derived sensory neurons at plasma equivalent concentrations (50 µM).

Based on this preclinical prediction the patient started off-label treatment with lacosamide. Within five days pain ratings on numeric rating scale (0 no pain, 10 worst imaginable pain) decreased from 7.5 to 1.5. Simultaneously spontaneous activity of C-fibers in microneurography recordings was diminished to 7.1%, proving that lacosamide is also effective in the peripheral nervous system. When the patient interrupted medication due to increased sedating side effects by combining lacosamide with antiallergic medication during hay fever season, this interrupti-

Prof. Dr. Winner Prof. Dr. Schüttler



Lacosamide reduced spontaneous activity of patient-derived nociceptors in MEA recordings (left panel). In a subsequent individualised therapeutic approach lacosamide decreased activity of patient C-fibers and alleviated the patient's pain (right panel).

on of lacosamide treatment led to reoccurrence of severe pain. After continuing lacosamide pain levels again decreased to NRS 1.5 and the effect was still preserved after 6 month of treatment.

In summary our findings make a mere placebo effect of lacosamide seem unlikely and provide evidence for an individualized translational therapeutic approach based upon patient-derived sensory neurons which could be confirmed to revert pathology in patient's peripheral neurons. This is an example of successful patient specific precision medicine using iPSC technology and individualized therapeutic treatment based on patient-derived sensory neurons

Contact:
Prof. Dr. Winner
phone: +49 9131 85 39301
e-mail: beate.winner@fau.de

Publications during funding period

Turan S, Boerstler T, Kavyanifar A, Loskarn S, Reis A, Winner B, Lie DC (2019) A novel human stem cell model for Coffin-Siris Syndrome like syndrome reveals the importance of SOX11 dosage for neuronal differentiation and survival. Hum Mol Genet. doi: 10.1093/hmg/ddz089

Pozner T, Schray A, Regensburger M, Lie DC, Schlötzer-Schrehardt U, Winkler J, Turan S, Winner B (2018) Tideglusib Rescues Neurite Pathology of SPG11 iPSC Derived Cortical Neurons. Front Neurosci 12:914

Namer B, Schmidt D, Eberhardt E, Maroni M, Dorfmeister E, Kleggetveit IP, Kaluza L, Meents J, Gerlach A, Lin Z, Winterpacht A, Dragicevic E, Kohl Z, Schüttler J, Kurth I, Warncke T, Jorum E, Winner B, Lampert A (2019) Pain relief in a neuropathy patient by lacosamide: Proof of principle of clinical translation from patient-specific iPS cell-derived nociceptors. EBioMedicine 39:401-408

Kaindl J, Meiser I, Majer J, Sommer A, Krach F, Katsen-Globa A, Winkler J, Zimmermann H, Neubauer JC, Winner B (2019) Zooming in on Cryopreservation of hiPSCs and Neural Derivatives: A Dual-Center Study Using Adherent Vitrification. Stem Cells Transl Med 8:247-259

Popp B, Krumbiegel M, Grosch J, Sommer A, Uebe S, Kohl Z, Plötz S, Farrell M, Trautmann U, Kraus C, Ekici AB, Asadollahi R, Regensburger M, Günther K, Rauch A, Edenhofer F, Winkler J, Winner B, Reis A (2018) Need for high-resolution Genetic Analysis in iPSC: Results and Lessons from the ForIPS Consortium. Sci Rep 8:17201

Schlachetzki JCM, Prots I, Tao J, Chun HB, Saijo K, Gosselin D, Winner B, Glass CK, Winkler J (2018) A monocyte gene expression signature in the early clinical course of Parkinson's disease. Sci Rep 8:10757

Prots I, Grosch J, Brazdis RM, Simmnacher K, Veber V, Havlicek S, Hannappel C, Krach F, Krumbiegel M, Schütz O, Reis A, Wrasidlo W, Galasko DR, Groemer TW, Masliah E, Schlötzer-Schrehardt U, Xiang W, Winkler J, Winner B (2018) a-Synuclein oligomers induce early axonal dysfunction in human iPSC-based models of synucleinopathies. Proc Natl Acad Sci USA 115:7813-7818

Schulze M, Sommer A, Plötz S, Farrell M, Winner B, Grosch J, Winkler J, Riemenschneider MJ (2018) Sporadic Parkinson's disease derived neuronal cells show disease-specific mRNA and small RNA signatures with abundant deregulation of piRNAs. Acta Neuropathol Commun 6:58

Sommer A, Marxreiter F, Krach F, Fadler T, Grosch J, Maroni M, Graef D, Eberhardt E, Riemenschneider MJ, Yeo GW, Kohl Z, Xiang W, Gage FH, Winkler J, Prots I, Winner B (2018) Th17 Lymphocytes Induce Neuronal Cell Death in a Human iPSC-Based Model of Parkinson's Disease. Cell Stem Cell 23:123-131

Regensburger M, Prots I, Reimer D, Brachs S, Loskarn S, Lie DC, Mielenz D, Winner B (2018) Impact of Swiprosin-1/Efhd2 on Adult Hippocampal Neurogenesis. Stem cell reports 10:347-355

E26 - Final Report

01/03/2016 - 28/02/2019

Genetics and pathomechanisms of intellectual disability with microcephaly

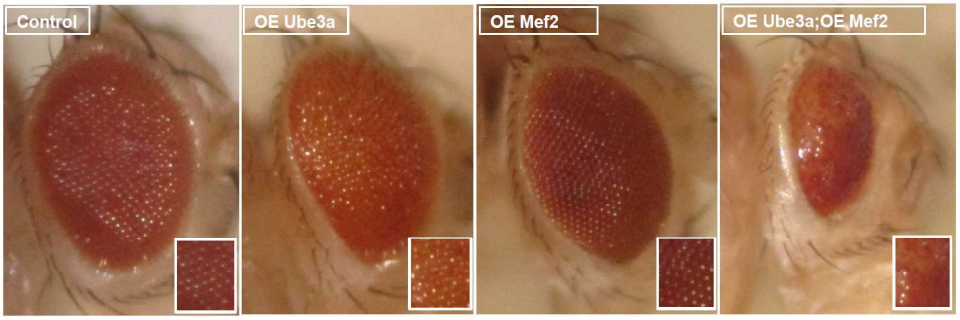
Prof. Dr. Dr. Christiane Zweier, Institute of Human Genetics

Mutations in genes from the same pathway often result in overlapping clinical phenotypes. Thus, co-morbidity of postnatal microcephaly with intellectual disability (ID) can indicate a genetic defect affecting neuronal migration, apoptosis or dendrite and synapse formation. We aim at the identification of novel, underlying genes in a group of patients with postnatal microcephaly and ID and to characterize their roles and interactions within common pathways and biological processes.

We selected five genes (TCF4, MEF2C, UBE3A, ZEB2 and ATRX) implicated in clinically overlapping, syndromic forms of severe ID with epilepsy and postnatal microcephaly that are often considered as close differential diagnoses. Most of these genes are involved in transcriptional regulation. By using genome-wide transcriptome analysis and by using Drosophila melanogaster as a model to screen for genetic interactions, we identified commonly deregulated target genes involved in neurodevelopment and specific genetic interactions, e.g. between Ube3a and Mef2. These molecular commonalities might contribute to the clinically overlapping features of the investigated disorders.

Genetic Interaction screen in Drosophila demonstrates functional links between ID genes

We assessed potential genetic interactions of orthologues of the five ID genes in Drosophila melanogaster. We used the UAS/GAL4 system to induce knockdown or overexpression of each single gene and in pairwise combinations ubiquitously and in various specific tissues (eye, wing, pan-neuronal, glia). Modification of a phenotype resulting from deregulation of a single gene A by simultaneous deregulation of a second gene B indicates genetic interaction between gene A and B. Several parameters such as lethality, wing and eye morphology, neuromuscular junction morphology and bang sensitivity and climbing behaviour were assessed. We found evidence for genetic interaction between several of these genes, most stringently between Ube3a and Mef2: pairwise knockdown and pairwise overexpression as well as a combination of Ube3a-overexpression and Mef2-knockdown resulted in either more severe or milder wing and eye phenotypes compared to single knockdown or overexpression of any of the two genes.

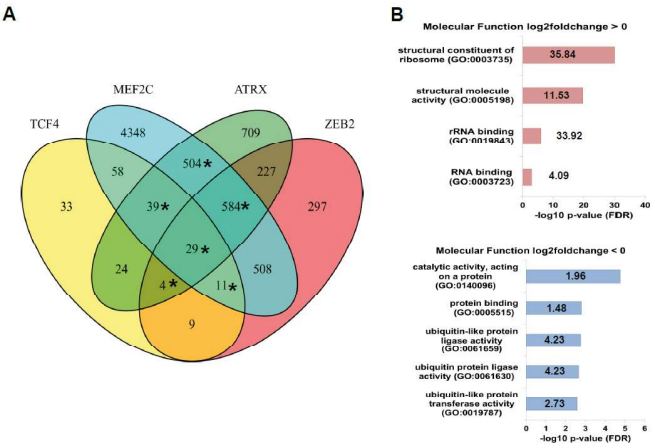


Genetic Interaction. While overexpression of Ube3a or Mef2 alone causes a mild eye phenotype (rough eye or no bristles), respectively, simultaneous overexpression of both genes results in a severely reduced eye size and dissolved ommatidia structure.

Prof. Dr. Dr. Zweier

Transcriptome analysis identifies commonly deregulated target genes

We performed transcriptome analysis on RNA from patient blood samples to investigate possible common transcriptional targets of the four transcriptional regulators TCF4, ZEB2 (3 individuals, each), MEF2C, and ATRX (1 individual, each). In all patient groups, markedly more genes were down-regulated than up-regulated (TCF4: 132 vs. 75, ZEB2: 1163 vs. 506, MEF2C: 3779 vs. 2311, ATRX: 1505 vs. 615), indicating a shared role as transcriptional activators for all four proteins. Pairwise comparison of deregulated genes in the four patient groups revealed significant overlap, indicating common transcriptional targets. Moreover, we found significant enrichment of known ID genes among the deregulated genes, indicating central roles of the four proteins in neurodevelopment. 667 genes were commonly deregulated in at least three of the four patient groups. These genes were enriched for gene ontology terms such as ribosomal and RNA-related functions (up-regulated genes) and catalytic activity ubiquitin ligase activity (down-regulated genes).



Transcriptome Analysis. A: Overlap of deregulated genes in patients with mutations in one of the four tested genes. B: Gene Ontology Term enrichment in commonly deregulated genes (marked by * in A).

Contact:
Prof. Dr. Dr. Zweier
phone: +49 9131 85 29113
e-mail: christiane.zweier@uk-erlangen.de

Publications during funding period

Kraus C, Uebe S, Thiel CT, Ekici AB, Reis A, Zweier C (2018) Microphthalmia is not a mandatory finding in X-linked recessive syndromic microphthalmia caused by the recurrent BCOR variant p.Pro85Leu. Am J Med Genet A 176: 2872-2876

Gregor A, Sadleir LG, Asadollahi R, Azzarello-Burri S, Battaglia A, Ousager LB, Boonsawat P, Bruel AL, Buchert R, Calpena E, Cogné B, Dallapiccola B, Distelmaier F, Elmslie F, Faivre L, Haack TB, Harrison V, Henderson A, Hunt D, Isidor B, Joset P, Kumada S, Lachmeijer AMA, Lees M, Lynch SA, Martinez F, Matsumoto N, McDougall C, Mefford HC, Miyake N, Myers CT, Moutton S, Nesbitt A, Novelli A, Orellana C, Rauch A, Rosello M, Saida K, Santani AB, Sarkar A, Scheffer IE, Shinawi M, Steindl K, Symonds JD, Zackai EH; University of Washington Center for Mendelian Genomics; DDD Study, Reis A, Sticht H, Zweier C (2018) De Novo Variants in the F-Box Protein FBXO11 in 20 Individuals with a Variable Neurodevelopmental Disorder. Am J Hum Genet 103:305-316

Jung M, Häberle BM, Tschaikowsky T, Wittmann MT, Balta EA, Stadler VC, Zweier C, Dörfler A, Gloeckner CJ, Lie DC (2018) Analysis of the expression pattern of the schizophrenia-risk and intellectual disability gene TCF4 in the developing and adult brain suggests a role in development and plasticity of cortical and hippocampal neurons. Mol Autism 9: 20

Straub J, Konrad EDH, Grüner J, Toutain A, Bok LA, Cho MT, Crawford HP, Dubbs H, Douglas G, Jobling R, Johnson D, Krock B, Mikati MA, Nesbitt A, Nicolai J, Phillips M, Poduri A, Ortiz-Gonzalez XR, Powis Z, Santani A, Smith L, Stegmann APA, Stumpel C, Vreeburg M; Deciphering Developmental Disorders Study, Fliedner A, Gregor A, Sticht H, Zweier C (2018) Missense Variants in RHOBTB2 Cause a Developmental and Epileptic Encephalopathy in Humans, and Altered Levels Cause Neurological Defects in Drosophila. Am J Hum Genet 102: 44-57

Popp B, Ekici AB, Thiel CT, Hoyer J, Wiesener A, Kraus C, Reis A, Zweier C (2017) Exome Pool-Seq in neurodevelopmental disorders. Eur J Hum Genet 25: 1364-1376

Reuter MS, Krumbiegel M, Schlüter G, Ekici AB, Reis A, Zweier C (2017) Haploinsufficiency of NR4A2 is associated with a neurodevelopmental phenotype with prominent language impairment. Am J Med Genet A 173: 2231-2234

Smogavec M, Cleall A, Hoyer J, Lederer D, Nassogne M-C, Palmer EE, Deprez M, Benoit V, Maystadt I, Noakes C, Leal A, Shaw M, Gecz J, Raymond L, Reis A, Shears D, Brockmann K, Zweier C (2016) Eight further individuals with intellectual disability and epilepsy carrying bi-allelic CNTNAP2 aberrations allow delineation of the mutational and phenotypic spectrum. J Med Genet 53: 820-27

Saunier C, Støve SI, Popp B, Gérard B, Blenski M, AhMew N, de Bie C, Goldenberg P, Isidor B, Keren B, Leheup B, Lampert L, Mignot C, Tezcan K, Mancini GM, Nava C, Wasserstein M, Bruel AL, Thevenon J, Masurel A, Duffourd Y, Kuentz P, Huet F, Rivière JB, van Slegtenhorst M, Faivre L, Piton A, Reis A, Arnesen T, Thauvin-Robinet C, Zweier C (2016) Expanding the Phenotype Associated with NAA10-Related N-Terminal Acetylation Deficiency. Hum Mutat 37: 755-64

E27 - Final Report

01/03/2016 - 28/02/2019

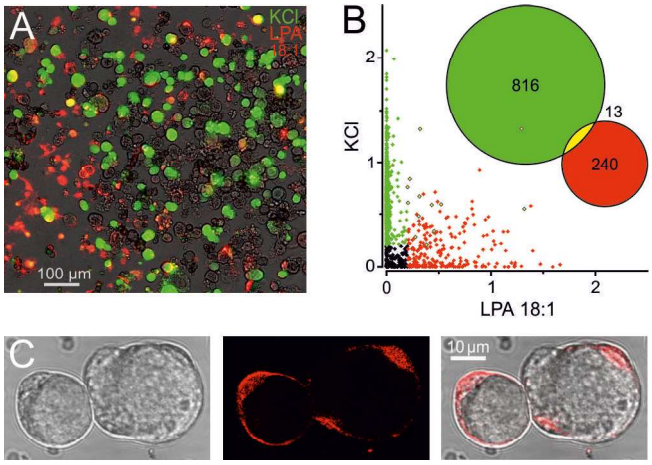
Lysophosphatidic acid-induced pruritus of cholestasis

PD Dr. Dr. Andreas Kremer, Department of Medicine 1 - Gastroenterology, Pneumology and Endocrinology
Prof. Dr. Michael Fischer, Institute of Physiology and Pathophysiology (till 31/08/2016)

In cholestatic patients with chronic pruritus we previously found elevated serum levels of lysophosphatidic acid (LPA). The aim of this translational project is to unravel the molecular mechanisms of LPA in cellular assays and to understand the interaction with substances known to cause itch. This will be validated in an animal model and tested in preclinical human studies. Unravelling the signaling pathway could open new avenues for causal anti-pruritic treatment strategies.

Unravelling the LPA-signaling axis between glia cells and sensory neurons

LPA and known agonists were sequentially applied to dissociated mouse dorsal root ganglia (DRG). LPA-activated cells differ from cells activated by potassium chloride (KCl), used as positive control for neurons. Cells responsive to both LPA and KCl were rare, as could be shown by comparing the respective calcium time courses and the inverse correlation between these responses ($r = -0.37$, $p < 0.001$, $n = 1237$). The subsequent application of LPA 1 µM, several establishes TRP-channel agonists and KCl 60 mM showed two distinct response patterns. Cells were activated either by LPA or by neuronal receptor agonists. The activation pattern was analyzed regarding the cell phenotype: Only 1.6% (13 of 829) of all cells reacting to LPA 18:1 were neurons based on their phenotype and response to KCl. Responses to potassium were larger in neurons compared to SGCs but LPA differentiates the two populations more clearly. The phenotype of LPA-activated cells in DRGs matched those of satellite glia cells, the cells were small and found in a halo-like shape around neurons. In stained DRGs sections we observerd a co-localization for LPAR1 and the glial marker glutamine synthetase while the LPAR1 expression in neurons is marginal at best.



LPA 18:1 activates satellite glia cells but only 1.6% of sensory neurons. A) Overlay of transmission image (gray-scaled) and the response to LPA (red) and to KCl (green). B) Scatterplot of the ratio increase for every cell for LPA and KCl. C) Confocal image of satellite glia cells responding to LPA.

LPA activated more hTRPV1-transfected HEK293t cells compared to untransfected controls exceeding a threshold of 0.2 ratio increase (25% vs 1% in hTRPV1, $p < 0.001$, Chi-square test). As the magnitude of the response was only 11% of capsaicin, this indicates a clearly detectable but limited activation compared to the gold standard agonist. The activation of DRG cells from TRPV1-deficient mice were similar to wild type controls. Experiments in calcium-free conditions show that the LPA-induced increase in cytoplasmatic calcium is derived from the endoplasmatic reticulum. In combination with LPA receptor expression results from DRGs and Schwann cells, pharmacological results indicate a signaling pathway through LPAR1. This result is supported by the elimination of LPA responses using the LPAR1 and LPAR3 antagonist Ki16425.

PD Dr. Dr. Kremer Prof. Dr. Fischer

LPA-mediated activation of sensory neurons in healthy volunteers and cholestatic patients.

LPA was applied intradermally by insertion of LPA-loaded heat-inactivated cowhage spicules in healthy volunteers. Control applications included histamine, capsaicin and the vehicle solution. The pain and itch intensities were quantified using a numeric rating scale. LPA applied into the skin using cowhage spicules induced a mild itch sensation compared to vehicle control (mean ± SEM; 1.4 ± 0.4 vs. 0.3 ± 0.2; $p<0.001$) lasting for several minutes. In contrast, intradermal injection of LPA caused a dose-dependent burning pain which occurred delayed compared to capsaicin. LPA hardly induced any flare reaction but a sensitization to heat. Responses to cold, mechanical and electrical stimuli remained unaltered.

Contact:
PD Dr. Dr. Kremer
phone: + 49 9131 85 45015
e-mail: andreas.kremer@uk-erlangen.de

Prof. Dr. med. Michael Fischer
phone: +43 (0)1 40160-31410
e-mail: michael.jm.fischer@meduniwien.ac.at

Invited lectures

DGVS Seminar Hepatologie, Berlin, 06.03.19, „Primär biliäre Cholangitis und immun-vermittlete Cholangiopathien“

International Liver Meeting, Wien, 06.04.19, “Histological outcomes with long-term obeticholic acid therapy“

Grand Round, 23.04.19, Universität Innsbruck, “Cholestase und Pruritus“

Pharmacon, Bundesapothekertagung, Meran, 27.05.19, “ Chronischer Pruritus – Ursachen und Therapie“

China Lecture Tour, 06/19, Xi’an, Nanjing, Foshan, u.a., „NASH: the new epidemic in hepatology”

DGVS Tagung, Wiesbaden, 03.10.19, “PBC: Obeticholsäure versus Bezafibrat“

Publications during funding period

Dhillon AK, Kremer AE, Kummen M, Boberg KM, Elferink RPO, Karlsen TH, Beuers U, Vesterhus M, Hov JR (2019) Autotaxin activity predicts transplant-free survival in primary sclerosing cholangitis. Sci Rep 9(1):8450

Robering JW*, Gebhardt L*, Wolf K, Kühn H, Kremer AE*, Fischer MJM* (2019) Lysophosphatidic acid activates satellite glia cells and Schwann cells. Glia 67(5):999-1012 *contributed equally

Schmid R*, Wolf K*, Robering JW, Strauß S, Strissel PL, Strick R, Rübner M, Fasching PA, Horch RE, Kremer AE, Boos AM, Weigand A (2018) ADSCs and adipocytes are the main producers in the autotaxin-lysophosphatidic acid axis of breast cancer and healthy mammary tissue in vitro. BMC Cancer 18(1):1273

Babes A, Ciotu CI, Hoff mann T, Kichko TI, Selescu T, Neacsu C, Sauer SK, Reeh PW, Fischer MJM (2017) Photosensitization of TRPA1 and TRPV1 by 7-dehydrocholesterol: implications for the Smith-Lemli-Opitz syndrome. Pain 158(12): 2475-2486

Mack K, Fischer MJM (2017) Disrupting sensitization of TRPV4. Neuroscience 352: 1-8

Schwarz MG, Namer B, Reeh PW, Fischer MJ (2017) TRPA1 and TRPV1 antagonists do not inhibit human acidosis-induced pain. J Pain 18(5): 526-534

He GW, Günther C, Kremer AE, Thonn V, Amann K, Poremba C, Neurath MF, Wirtz S, Becker C (2017) PGAM5-mediated programmed necrosis of hepatocytes drives acute liver injury. Gut 66(4): 716-723

Günther C, He GW, Kremer AE, Murphy JM, Petrie EJ, Amann K, Vandenabeele P, Linkermann A, Poremba C, Schleicher U, Dewitz C, Krautwald S, Neurath MF, Becker C, Wirtz S (2016) The pseudokinase MLKL mediates programmed hepatocellular necrosis independently of RIPK3 during hepatitis. J Clin Invest. 126(11): 4346-4360

Wunsch E, Krawczyk M, Milkiewicz M, Trottier J, Barbier O, Neurath MF, Lammert F, Kremer AE*, Milkiewicz P* (2016) Serum Autotaxin is a Marker of the Severity of Liver Injury and Overall Survival in Patients with Cholestatic Liver Diseases. Sci Rep. 6: 30847 *contributed equally

Kühn H, Kappes L, Wolf K, Gebhardt L, Neurath MF, Reeh P, Fischer MJM, Kremer AE (2020) Complementary roles of murine NaV1.7, NaV1.8 and NaV1.9 in acute itch signaling. Sci Rep. 10: 2326

Newly started Projects

E28 30 months

Neural Crest Regulators In Orofacial Clefting


Prof. Dr. Gölz


Prof. Dr. Wegner

Prof. Dr. Lina Gölz,
Department of Orthodontics and Orofacial Orthopedics,
Prof. Dr. Michael Wegner, Institute of Biochemistry

Orofacial clefts are frequent congenital malformations. Etiology is complex, poorly understood and involves environmental and genetic factors. We have identified several cranial neural crest transcription factors and chromatin remodelers as key regulators of palatal development. We will use genome-edited cell lines and mouse mutants to determine the exact function and relationship of these factors in their regulatory network and thus better understand palatal development and orofacial clefting.

e-mail: lina.goelz@uk-erlangen.de

E29 01/01/2020 - 30/06/2022

The impact of lysosome dysfunction on stem cell ageing


Prof. Dr. Lie

Prof. Dr. Dieter Chichung Lie, Institute of Biochemistry

Recent data indicates that adult neural stem cell dysfunction and the resulting impairment of adult hippocampal neurogenesis contributes to cognitive deficits in human ageing and neurodegenerative diseases. The mechanisms underlying ageing-associated neural stem cell dysfunction are largely unknown. This project will investigate the hypothesis that dysfunction of lysosome-dependent degradation pathways is a major contributor to hippocampal neural stem cell dysfunction during ageing.

e-mail: dieter-chichung.lie@fau.de

E30 01/04/2020 - 30/09/2020

Impact of the immune system on Parkinson‘s disease


Prof. Dr. Winner


Prof. Dr. Winkler

Prof. Dr. Beate Winner, Department of Stem Cell Biology,
Prof. Dr. Jürgen Winkler, Department of Molecular Neurology

Recent data demonstrate profound immunological alterations in Parkinson’s disease (PD). We study the contribution of the peripheral immune system to onset and progression in PD. Specifically, we perform a comprehensive characterization of peripheral immunity in early vs. late onset with rapid vs. slow disease progression PD patients. Subsequently, we will determine neurotoxicity in human autologous co-cultures of stem cell-derived midbrain neurons and specific immune cells.

e-mail: beate.winner@uk-erlangen.de

E31 01/01/2020 - 30/06/2022

Proteasomal degradation in intellectual disability


Prof. Dr. Dr. Zweier

Prof. Dr. Dr. Christiane Zweier, Institute of Human Genetics

Neurodevelopmental disorders (NDDs) are extremely heterogeneous but converge on a number of common molecular processes. Treatment options are limited so far. We will focus on a subset of NDD associated genes/proteins which are involved in the ubiquitin-proteasome system. We will investigate if manipulation of proteasome activity by small molecules can ameliorate phenotypes in Drosophila and/or cell based model systems and thus will gain insights into potential interventional options.

e-mail: christiane.zweier@uk-erlangen.de

F6 - Final Report

01/07/2016 - 30/06/2019

Renal afferent nerve activity - sympathoinhibitory or sympathoexcitatory?

Prof. Dr. Roland Veelken, Department of Medicine 4
Prof. Dr. Kerstin Amann, Department of Nephropathology

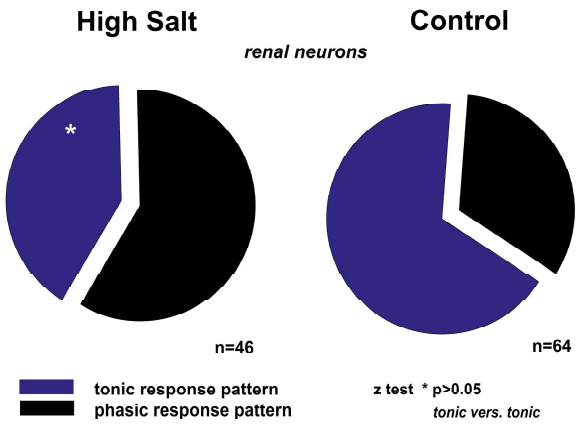
All our experimental activities during the time of the project always supported our main hypothesis that afferent nerves from the kidneys are important cardiovascular regulators in health and disease. The last period of the funded project focused on the question of what influence a high salt intake might have on afferent peptidergic nerve fibers.

Renal afferent nerve fibers – alterations during in sodium diet ?

We had previously seen that superperfusion of cultured neurons with afferent nerve fibers from the kidney with saline induced action potential productions that decreased in response to higher NaCl concentrations. When we now cultivated respective renal neurons from rats that had been on a high salt diet for ten days, it turned out that the number of highly active tonic neurons with afferent renal projections, whose high proportion in the afferent autonomic innervation is typical of the kidney, had significantly decreased. At the same time, the remaining highly active neurons showed an increased compensatory maximum activity upon stimulation. Ongoing in vivo experiments suggest that this compensatory increase in activity of afferent nerve units from the kidney is not sufficient to compensate for the reduction in highly active afferent neurons. The inhibition of renal sympathetic activity via afferent nerve fibers is probably reduced in rats on a high salt diet compared to control animals. These findings indicate that high salt intake limits the control of sympathetic nerve activity, whereby the involved afferent nerve fibres obviously exert tonic sympathetic inhibition under normal conditions, which is lost with high salt intake

Neurogenic CGRP in the Skin and High Sodium Diet

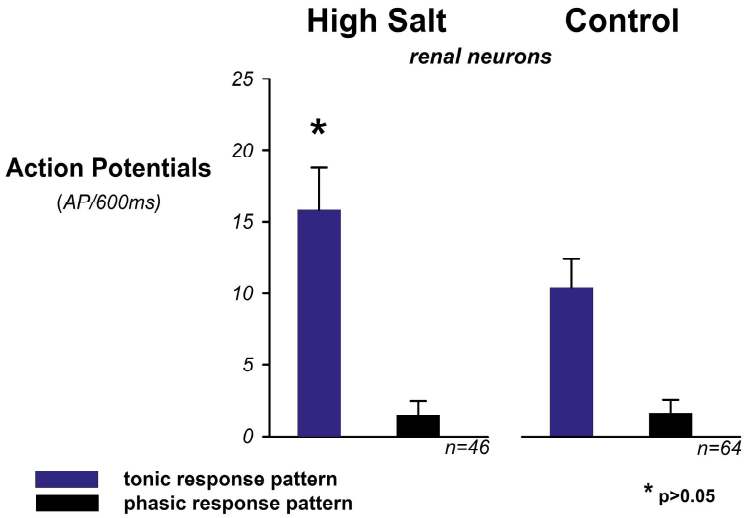
It has been known for some time that sodium probably will be also stored osmotically non-active in the skin. A high-salt diet in rats had led to interstitial hypertonic sodium accumulation in the skin, resulting in increased density and hyperplasia of the lymphocapillary network. Since the skin is the organ that has the highest number of afferent peptidergic nerve fibers, i.e. fibers that can potentially release their vasoactive peptide CGRP, it was hypothesized that these fibers could be involved in the non-osmotic storage of sodium in the skin. (The only significant source of CGRP in the skin are afferent nerve fibers). It seemed conceivable that an increased cutaneous microcirculation due to neurogenic CGRP release might lead to an increased formation of lymph vessels that serve the non-osmotic storage of NaCl. In wound healing processes, even a role of CGRP in



The ratios of highly active tonic to less active phasic neurons with afferent nerve fibers from the kidney are shown. The number of highly active nerve fibers decreased dramatically for animals fed a high salt diet.

Prof. Dr. Veelken

Prof. Dr. Amann



The remaining highly active neurons of the animals fed a high salt diet increased their maximum activity in a compensatory manner

lymphangiogenesis was described. We could that rats on a high salt diet showed a significantly higher release from skin nerves after ten days of high salt. However, the action potential production of the corresponding neurons with afferent nerve fibers from the skin was not altered, indicating normal sensory performance and nociception. Peptidergic afferent nerve fibers could thus form an integrated, body-wide system involved in sodium metabolism in very different target areas such as skin and kidney.

Contact:
Prof. Dr. Veelken
phone: +49 9131 85 39201
e-mail: roland.veelken@uk-erlangen.de

Prof. Dr. Amann
phone: +49 9131 85 22291
e-mail: kerstin.amann@uk-erlangen.de

Invited lectures

PD Dr. med. Tilman Ditting, Jahrestagung der deutschen Gesellschaft für Nephrologie, Oktober 10-13, 2019, Düsseldorf

Awards

Best Abstract, Dr. med Kristina Rodionova, Jahrestagung der deutschen Gesellschaft für Nephrologie, Oktober 10-13, 2019, Düsseldorf

Publications during funding period

none

Newly started Projects

F7 30 months

Gpr126 (Adgrg6) in kidney development and disease


Prof. Dr. Engel

Prof. Dr. Felix Engel, Department of Nephropatology

Chronic kidney disease represents the fastest growing pathology worldwide. Elucidating new regulators of kidney development and disease will promote the development of strategies for kidney repair. Here we propose to identify how the adhesion G protein-coupling receptor Gpr126 regulates kidney development and which diseases are associated with altered Gpr126 expression in order to design in the future experiments to determine whether Gpr126 inhibition or activation can improve kidney function.

e-mail: felix.engel@uk-erlangen.de

F8 01/02/2020 - 31/07/2022

Ion channel function of polycystin-2 in ADPKD


Prof. Dr. Korbmacher

Prof. Dr. Christoph Korbmacher, Institute of Cellular and Molecular Physiology

In about 15 % of affected patients ADPKD (autosomal dominant polycystic kidney disease) is caused by mutations in the PKD2 gene coding polycystin-2 (PC2). Altered ion channel properties of PC2 may contribute to the pathophysiology of ADPKD. This project uses a novel experimental strategy to study the electrophysiological properties of PC2 and mutant PC2 channels in combination with molecular modelling. Its aim is to improve our understanding of PC2 ion channel function in health and disease.

e-mail: christoph.korbmacher@fau.de

F9 01/04/2020 - 30/09/2022

Generation of novel glomerular 3D culture systems

PD Dr. Müller-Deile Prof. Schiffer

PD Dr. Janina Müller-Deile, Prof. Dr. Mario Schiffer,
Department of Medicine 4 – Nephrology and Hypertension

We will generate glomerular-like structures by co-culturing human glomerular cells ex vivo and use this model to study glomerular functions and cell-cell interactions. Podocytes with specific knockdown of podocyte genes in these co-cultures enable us to investigate the pathology in the context of all other glomerular cells. Using podocytes derived from patient urines allows to characterise renal disease on a personalised level and to study therapeutic substances for the individual patient.

e-mail: janina.mueller-deile@uk-erlangen.de

Junior Research Group 1

Prof. Dr. Paolo Ceppi

Contact:
phone: +49 9131 85 39300
e-mail: paolo.ceppi@uk-erlangen.de

Biographical Sketch

Prof. Dr. Paolo Ceppi started his appointment at the Interdisciplinary Center for Clinical Research (IZKF), Friedrich-Alexander University Erlangen-Nürnberg in Erlangen on August 1st, 2015.

Since August 2019 Paolo Ceppi is also an Associate Professor at the Department of Biochemistry and Molecular Biology (BMB) at the University of Southern Denmark in Odense.

Below is a list of the previous research appointments:

Mar 2011 – Jun 2015 Postdoctoral fellow at the Division of Hematology/Oncology, Feinberg School of Medicine, Robert H. Lurie Comprehensive Cancer Center Northwestern University, Chicago, USA (Prof. M. Peter).

Feb 2009 – Dec 2009 Visiting PhD student at the Department of Experimental Surgery and Molecular Oncology of Solid Tumors, Medical Faculty Mannheim, University of Heidelberg and DKFZ Heidelberg, Germany (Prof. H. Allgayer).

Jan 2007 – Dec 2010 PhD student in the Pathology Division of the Department of Clinical and Biological Sciences, University of Turin, Italy (Prof. M. Papotti).

Jul 2004 – Dec 2006 Research assistant at Thoracic Oncology Unit and the Pathology Division of the Department of Clinical and Biological Sciences, University of Turin, Italy (Prof. G. Scagliotti and Prof. M. Papotti).

Dec 2004 – Jun 2005 Visiting Research scholar at Department of Biochemistry and Molecular Biology, Norris Cancer Center, University of Southern California, Los Angeles, USA (Prof. P. Danenberg).

Top row from the left: Vignesh Ramesh, Paradesi Naidu Gollavilli, Aarif Siddiqui, Heike Wagner
Bottom row from the left: Annemarie Schwab, Beatrice Parma, Sabine Marschall, Paolo Ceppi

Research Focus

The theme of the Junior Group 1 is „Understanding the plasticity of cancer cells".

Background and Rationale: Despite the progresses made in the last years with the development of novel molecularly targeted agents, cancer is still a very deadly disease. This could be attributable in part to the fact that only a minority of selected patients benefit from the novel compounds (such as those targeting oncogenic drivers like EGFR, BRAF, HER2 and many others), while poor therapeutical options are available for the vast majority of the patients in which a targetable driving oncogenic mutation is undetermined. Moreover, the pathway redundancy and the very frequent occurrence of mutations limit the efficacy of these novel drugs even in initially responding patients. There is therefore an urgent need for the identification of novel fundamental mechanisms of cancer biology and of relevant determinants of chemoresistance in order to develop more effective drugs and therapeutic strategies.

The discovery of epithelial-to-mesenchymal transition (EMT), cancer stem cells (CSCs) and of their functional association and interdependence represent some of the most promising advances in the last two decades of cancer research. CSCs are defined as a subpopulation of undifferentiated cancer cells with stem-like features responsible for tumors' heterogeneity and for some of the most lethal features of cancers: tumorigenicity, metastatic spread, relapse and chemoresistance. The inter-conversion between CSCs and non-CSCs has been recently reported and the EMT clearly functionally involved. The EMT is a de-differentiation process frequently observed in cancers with increased invasive potential and drug resistance. A recently emerging concept is that the plasticity of cancers is greater than what initially hypothesized, and therefore a better understanding of the mechanisms behind the inter-conversion of cancer cells between differentiation stages may have many therapeutic implications. Moreover, cancers, and the CSC population in particular, are highly dependent on aerobic glycolysis, which they use as a major pathway for biosynthesis. The enhanced rate of glycolysis occurs largely because of the increased demand of a transformed cell for macromolecule components (the so-called Warburg effect). The connection between increased glycolytic rate, EMT and CSCs has recently started to emerge in the literature, but the molecular determinants involved are still undefined.

Aim of the research: The Junior Group aims at discovering fundamental druggable mechanisms and molecular determinants that regulate the plasticity and the aggressiveness of cancer cells, and at studying the association between cancer differentiation and sensitivity to chemotherapy. By high-throughput approaches we have identified a number of potential EMT/CSC-regulating metabolic mechanisms, which we aim to validate by the analysis of human samples and functionally investigate by the use of cell and molecular biology techniques. This approach may ultimately lead to the identifications of novel targets for therapeutic intervention.

Third-party funding

Paolo Ceppi, German Cancer Aid Research Grant, Determination of the role of aldose reductase AKR1B1 and associated pathways in epithelial-to-mesenchymal transition and cancer stem cells (2017-2020),

Paolo Ceppi, International Association for the Study of Lung Cancer. The role of thymidylate synthase in epithelial-to-mesenchymal transition in NSCLC (2017-2018),

Paolo Ceppi, DFG Research Grant. Whole-genome CRISPR/Cas9 mediated identification of miR-200 repressors (2018-2021),

Paolo Ceppi, DFG Research Grant. Deciphering and targeting the metabolic control of lung cancer de-differentiation (2019-2022).

N1 - Progress Report

01/08/2015 - 31/07/2021

Understanding the plasticity of cancer cells

Prof. Dr. Paolo Ceppi, IZKF - Junior Research Group 1

The group focuses on the identification of novel fundamental mechanisms of cancer biology using several cell and molecular biology techniques, mouse models, high-throughput approaches and the analysis of human samples. We aim at discovering novel genes and molecular pathways that regulate the plasticity and the aggressiveness of cancer cells and at studying the association between cancer differentiation and sensitivity to chemotherapy, with a special attention on metabolism genes. The final goal is the development of more effective drugs and therapeutic strategies.

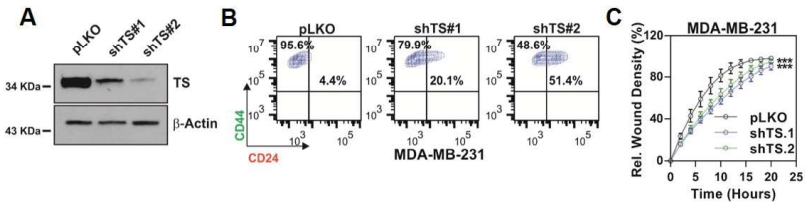
The activity of the lab during the reported period has been mainly focused on exploring the role of nucleotide metabolism in triple negative breast cancers:

Summary: Cancer cells frequently boost nucleotide metabolism (NM) to support their increased proliferation, but the consequences of elevated NM on tumor de-differentiation are mostly unexplored. We identified a role for thymidylate synthase (TS), a NM enzyme and established drug target, in cancer cell de-differentiation and investigated its clinical significance in breast cancer (BC).

In vitro, TS knockdown increased the population of CD24+ differentiated cells, and attenuated migration and sphere-formation. RNA-seq profiling indicated a repression of epithelial-to-mesenchymal transition (EMT) signature genes upon TS knockdown, and TS-deficient cells showed an increased ability to invade and metastasize in vivo, consistent with the occurrence of a partial EMT phenotype. Mechanistically, TS enzymatic activity was found essential for the maintenance of the EMT/stem-like state by fueling a dihydropyrimidine dehydrogenase – dependent pyrimidine catabolism. In patient tissues, TS levels were found significantly higher in poorly differentiated and in triple negative BC, and strongly correlated with worse prognosis.

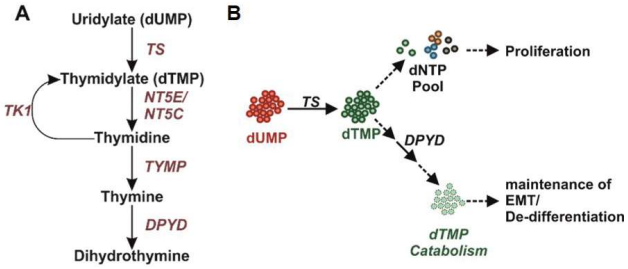
Taken together, these data contribute to identify an unprecedented non-proliferative role for TS and pyrimidine metabolism in cancer, providing the rationale to study in-depth the role of NM at the crossroads of proliferation and differentiation. Furthermore, this study may inspire the development of novel drug combinations for the treatment of triple negative breast cancers.

Reference:
Siddiqui A, Gollavilli P, Schwab A, Vazakidou ME, Ersan PG, Ramakrishnan M, Pluim D, Coggins SA, Saatci O, Annaratone L, Schellens JHM, Kim B, Asangani IA, Rasheed SAK, Marchiò C, Sahin O, Ceppi P. Thymidylate synthase maintains the de-differentiated state of aggressive breast cancers. Cell Death and Differentiation 2019 Feb 8. DOI: 10.1038/s41418-019-0289-6.



Thymidylate synthase (TS) suppression inhibits the cancer stem cell phenotype and migration in triple negative breast cancer. A) Western blot, B) FACS staining on CD44/CD24 and C) Incucyte migration assay in MDA-MB-231 cells infected with shRNAs targeting TS compared to scrambled-infected cells.

Prof. Dr. Ceppi



Scheme of the proposed role of TS in driving the de-differentiated phenotype of triple negative breast cancer. Excess thymidylate (dTMP) which is not used as a precursor for DNA synthesis is degraded in the catabolic pathway (A) by the DPYD enzyme supporting the EMT/CSC state (B).

We are also in the process of writing a Review article on the non-proliferative role of pyrimidine metabolism in cancer, which will be soon submitted for publication.

In addition, another project has been completed in the reported year, in collaboration with Dr. Federico Cerrone and Prof. Kevin O'Connor from the School of Biomolecular and Biomedical Science of the University College Dublin in Ireland, and with the Winner lab at the Uniklinikum Erlangen. The project is entitled "Electrospun nanofibres of bacterially synthesized aromatic polyesters prolong the life-span of human IPSC-derived cortical neuronal cells" and it is currently submitted for publication.

Contact:
Prof. Dr. Ceppi
phone: +49 9131 85 39300
e-mail: paolo.ceppi@uk-erlangen.de

Invited lectures

Seminar series of the Zentralinstitut für Medizintechnik (ZiMT), January 14th 2019 in Erlangen. Title: Metabolic pathways as regulators of EMT.

5th Stem Cells and Regenerative Medicine Meeting organized by the International Society for Cell and Gene Therapy, October 22nd 2019 in Mexico City, Mexico. Title: Thymidylate synthase maintains the EMT/CSCs-like state of TN breast cancer.

Seminar series of the Institute of Biomedical Investigations of the Faculty of Medicine, National Autonomous University of Mexico (UNAM), October 24th 2019 in Mexico City, Mexico. Title: Metabolic pathways and miRNAs as regulators of EMT.

Publications during funding period

Siddiqui A, Gollavilli P, Schwab A, Vazakidou ME, Ersan PG, Ramakrishnan M, Pluim D, Coggins SA, Saatci O, Annaratone L, Schellens JHM, Kim B, Asangani IA, Rasheed SAK, Marchiò C, Sahin O, Ceppi P (2019) Thymidylate synthase maintains the de-differentiated state of aggressive breast cancers. Cell Death and Differentiation 26(11):2223-2236

Krumbholz M, Woessmann W, Zierk J, Seniuk D, Ceppi P, Zimmermann M, Singh V, Metzler M, Damm-Welk C (2018) Characterization and diagnostic application of genomic NPM-ALK fusion sequences in anaplastic large-cell lymphoma. Oncotarget 9(41):26543-26555

Schwab A, Siddiqui A, Vazakidou ME, Napoli F, Böttcher M, Menchicchi B, Raza U, Saatci Ö, Krebs AM, Ferrazzi F, Rapa I, Dettmer-Wilde K, Waldner MJ, Ekici AB, Rasheed SAK, Mougiakakos D, Oefner PJ, Sahin Ö, Volante M, Greten FR, Brabletz T, Ceppi P (2018) Polyol pathway links glucose metabolism to the aggressiveness of cancer cells. Cancer Research. 78:1604-1618.

Rasheed SAK, Leong HS, Lakshmanan M, Raju A, Dadlani D, Chong FT, Rajarethinam R, Skanthakumar T, Tan EY, Hwang JSK, Lim KH, Tan DS, Ceppi P, Wang M, Tergaonkar V, Casey PJ, Iyer G (2018) GNA13 expression promotes drug resistance and tumor-initiating phenotypes in solid tumors. Oncogene 37(10):1340-1353

Siddiqui A, Vazakidou ME, Schwab A, Napoli F, Fernandez-Molina C, Rapa I, Stemmler MP, Volante M, Brabletz T, Ceppi P (2017) Thymidylate synthase is functionally associated with ZEB1 and contributes to the epithelial-to-mesenchymal transition of cancer cells. The Journal of Pathology. 242:221-233

Junior Research Group 2

Dr. David Dulin

Contact:
phone: +49 9131 85 70347
e-mail: david.dulin@uk-erlangen.de

Biographical Sketch

Since September 2016, Dr. Dulin has started the "Physics and Medicine" IZKF Junior Research group N2 at Erlangen, aiming at studying viral and cellular transcription and replication at the single-molecule level using biophysical techniques.

Before starting his lab, Dr. Dulin graduated his Bachelor in physics and mathematics at the University of Bordeaux (France) in 2004 and his Master "Laser, Matter and Nanoscience" in 2006.

Between 2006 and 2009, he was a PhD candidate in the Laboratory Charles Fabry of the Institut d'Optique (Paris) in the group of Prof. A. Aspect and under the supervision of Prof. N. Westbrook. There, he worked at establishing a new biophysics lab, with a focus on bacterial ribosome translation kinetics using single-molecule fluorescence microscopy.

He then moved to a first postdoctoral position in the lab of Prof. N. Dekker at TU Delft (The Netherlands), where he stayed until August 2014. There, he developed new magnetic tweezers approaches for high throughput and high-resolution study of polymerases and helicases kinetics. In particular, he studied the mechanism of misincorporation and antiviral nucleotide analogue incorporation by viral polymerase. He then moved to the University of Oxford (UK) for a second postdoctoral position, where he studied bacterial transcription initiation dynamics using single-molecule FRET in the lab of Prof. A. Kapanidis, until being appointed in Erlangen.

From the left: David Dulin, Ibrahim Obulqasim, Flavia Stal-Papini, Mona Seifert, Eugen Ostrofet, Subas Chandra Bera.

Research Focus

The Dulin lab aims at understanding the fundamental processes involved in the central dogma of molecular biology, i.e. replication, transcription and translation, using high-end microscopy. Each step in gene expression involves complex molecular motors, e.g. DNA polymerase, RNA polymerase (RNAP) and ribosome. Much has been learned related to these motors using standard ensemble biochemical assay, but their detailed kinetic characterization remains elusive. Indeed, these enzymes do not progress linearly along their template, but rather through burst of successive catalytic reactions interrupted by pauses of various origins, e.g. co-factors binding, misincorporation, template sequence, which makes gene expression highly stochastic, and impacts the organisms phenotype. By giving access to enzymatic processes at the single molecule level, and not to the ensemble population, single-molecule biophysics has changed our view on biology, offering an understanding of the rare, transient and stochastic — but important — events that interrupt enzymatic activity. Our lab develops high-end microscopy techniques, such as magnetic tweezers and single-molecule fluorescence microscopy, to describe in great details (1) how RNA viruses replicate their genome and (2) how bacteria and eukaryotic cells transcribe their genomes.

1- RNA virus replication mechanism
RNA viruses represent an important class of human and animal pathogens. They are responsible of numerous pandemics worldwide, with an important economical and societal cost. Our lab is interested in interrogating how RNA viruses replicate their genome, and whether there are conserved mechanisms that could be targeted by antiviral drugs. In particular, our lab focuses its research on positive single stranded RNA ((+)ssRNA) virus, such as poliovirus and SARS-coronavirus. Poliovirus is a model system for the viral replication of (+)ssRNA virus and coronavirus such as SARS and MERS have a high lethality rate with no cure or vaccine existing. One key target for drug development is the replication machinery of these viruses. However, little is known concerning the mechanisms of genome replication for (+)ssRNA virus, limiting the potential development of drugs. Using magnetic tweezers and single-molecule fluorescence microscopy, we aim at understanding how the viral proteins that form the viral replicase are recruited and how they work in synergy during viral genome replication.

2- Cellular transcription
Transcription is at the heart of gene expression and maintenance any every organism. Our lab works on three different transcription systems: Escherichia coli (E. coli) bacteria, human mitochondria and yeast RNA polymerase I (Pol I). Bacterial transcription is the model system of cellular transcription and its most representative enzyme, E. coli RNA polymerase (RNAP), has been therefore intensively studied. We use the E. coli bacteria RNAP to benchmark our assays, and investigate the mechanisms of bacterial transcription initiation. Mitochondria are the powerhouse of the eukaryotic cell, and therefore, due to its importance in many cellular processes, abnormal mitochondria activity is linked to several disorders. Understanding the basis of mitochondria transcription, the first step in gene expression, will shed light on the biochemistry of this essential organelle. Pol I is responsible for synthesizing most of the ribosomal RNA, and is the rate limiting step in ribosome biogenesis. Because of its importance in ribosome production, thus on protein production, Pol I activity has become an attractive target for anti-cancer drugs. Using magnetic tweezers and single-molecule FRET assays, we investigate how these different RNA polymerases perform their transcription activity.

Third-party funding

David Dulin, DFG Research Grant, Revealing the mechanism of directional transcription termination at the single moleculelevel for the human mitochondrial transcription complex (2020-2022)

N2 - Progress Report

01/09/2016 - 31/08/2022

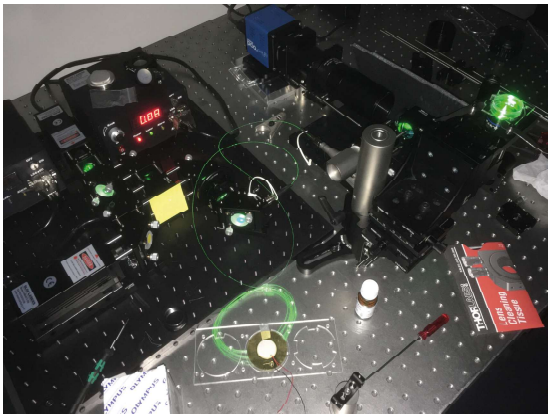
Physics and Medicine

Dr. David Dulin, IZKF - Junior Research Group 2

The Junior Group aims at understanding the molecular processes that regulate gene expression using high-end microscopy. We therefore develop single-molecule biophysics apparatuses to access enzymatic processes at the single-molecule level with high spatial (~nm) and temporal (~ms) resolution, to understand how nucleic acids are replicated and transcribed. In particular, we aim at understanding RNA virus genome replication and cellular transcription.

Since September 2016, the Junior Research Group N2 has grown to 6 members, including Dr. Flavia Stal-Papini (lab manager), Eugen Ostrofet (PhD candidate), Mona Seifert (PhD candidate), Ibrahim Obulqasim (PhD candidate), and Dr. Subhas Chandra Bera (Postdoc). In February 2019, the lab has moved to the Interdisciplinary Centre for Nanostructured Films (IZNF), Cauerstrasse 3 (south campus of the FAU at Erlangen), where we have relocated our microscopy and molecular biology laboratory.

To perform single-molecule magnetic tweezers experiments, it is necessary to design and synthesize specific nucleic acid scaffold. We have established several protocols to synthesize DNA and RNA scaffolds for the different experiments we perform in our lab, and we have published an article in Nucleic Acids Research that summarizes these developments. (Project leader: Dr. F. Stal Papini). Magnetic tweezers is a force spectroscopy technique, which necessitate precise calibration to extract useful information on the strength of interactions in a biomolecular complex. We have developed a new approach to perform such calibration, which has recently been published in Scientific Reports (Project leader: E. Ostrofet). Finally, we have also worked on improving the spatiotemporal resolution of magnetic tweezers, enabling single DNA base pair (0.34 nm) translocation observation in real-time and we have just submitted an article on this topic.

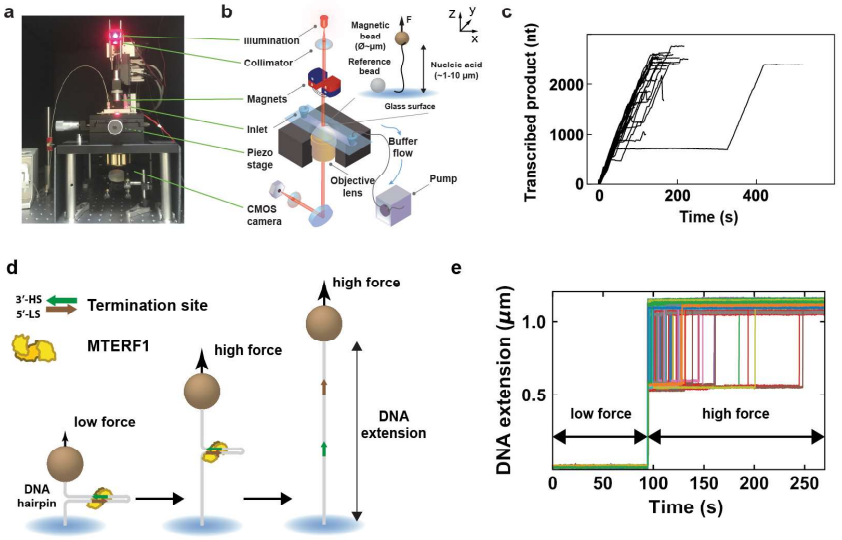


Two-colors total internal reflection fluorescence microscope (TIRFM) for single molecule FRET currently in development in the Dulin lab.

In addition, we have developed a simple and efficient temperature control system to perform experiments at a constant temperature up to ~50°C. This technique could easily be implemented to other types of high-end microscope. We used this assay to study the temperature dependence of the replication activity of poliovirus, human rhinovirus C and Φ6 RdRps, and we have just submitted a publication about this research (BioRxiv: https://doi.org/10.1101/2020.01.15.906032). We are now investigating the elongation kinetics of SARS-coronavirus replisome, a complex multi-factor replication factory in charge of synthesizing one of the largest viral RNA genome to be known, and how this virus eludes antiviral replication inhibitors (Project leader: M. Seifert).

We are also investigating the directionality and the mechanism of transcription termination in human mitochondria. In mitochondria, transcription is terminated in a directional manner by a protein coined

Dr. Dulin



(a) Magnetic tweezers set up and (b) its schematic description. (c) Traces of individual human rhinovirus C polymerase from a single experiment. (d) MTERF1-DNA interaction study using a force jump experiment on a DNA hairpin. (e) Experimental observations from the experiment described in (d): MTERF1 blocks the opening of the DNA hairpin.

MTERF1, which specifically binds to the termination site. To investigate how is triggered the transcription termination directionally, we have developed a DNA hairpin based approach. (Project leader: E. Ostrofet). Interestingly, this strategy for transcription termination is shared with Pol I, potentially presenting a conserved mechanism for termination induced by a DNA bound protein. We have further developed our cellular transcription endeavor by looking into the mechanisms of yeast Pol I and E. coli RNAP transcription initiation (Project leader: Dr. S.C. Bera), and we are currently preparing an article about the latter.

Finally, we are building a new high-end microscope (two-colors total internal reflection fluorescence microscope, TIRFM) to perform high spatiotemporal resolution fluorescence microscopy measurements. This instrument will be combined to magnetic tweezers in the near future to study cellular transcription and viral replication (Project leader: Ibrahim Obulqasim).

Contact:
Dr. Dulin
phone: +49 9131 85 70347
e-mail: david.dulin@uk-erlangen.de

Invited lectures

JFV2019 Annual Meeting of the French Society for Virology, March 2019, Lyon, France.

Single Molecule Biophysics meeting, January 2019, Aspen, CO, USA.

Publications during funding period

Papini FS, Seifert M, Dulin D (2019) High-yield fabrication of DNA and RNA constructs for single molecule force and torque spectroscopy experiments. Nucleic Acids Research, gkz851

Ostrofet E, Papini FS, Dulin D (2018) Correction-free force calibration for magnetic tweezers experiments. Scientific Reports 8:15920

Dulin D, Bauer DLV, Malinen AM, Bakermans JJW, Kaller M, Morichaud Z, Petushkov I, Depken M, Brodolin K, Kulbachinskiy A and Kapanidis AN (2018) Pausing controls branching between productive and non-productive pathways during initial transcription in bacteria. Nature Communications 9:1478

Kriegel F, Ermann N, Forbes R, Dulin D, Dekker NH, Lipfert J (2017) Probing the salt dependence of the torsional stiffness of DNA by multiplexed magnetic torque tweezers. Nucleic Acids Research, 45:5920-5929

Dulin D, Arnold JJ, van Laar T, Oh HS, Lee C, Harki DA, Depken M, Cameron CE, Dekker NH (2017) Signatures of Nucleotide Analogue Incorporation by an RNA-Dependent RNA Polymerase Revealed Using High-Throughput Magnetic Tweezers. Cell Reports, 21:1063-1076

J51 - Final Report

01/10/2015 - 31/03/2019

Neurosciences

Inflammatory signature in Parkinson´s disease

Dr. Franz Marxreiter, Department of Molecular Neurology

The project aims to address the contribution of inflammatory processes in the course of Parkinson's Disease (PD). During the funding period, we assessed the contribution of the innate immune system (specifically monocytes), and the adaptive immune system (specifically T-lymphocytes) in PD. Furthermore, we analyzed whether altered gut microbiota contribute to Parkinson's disease (PD). Here, we provide an overview of the results obtained during the funding period.

1. Innate Immune System (Monocytes)

Since previous studies suggested that an activation of the innate immune system may contribute to PD pathology (Hirsch & Hunot, 2009) we initially addressed the role of monocytes in PD. Monocytes have a critical role as effectors and regulators of the innate immune system. Therefore, we aimed to explore monocyte status as potential biomarker and/or regulator for PD. We were able to identify a distinct gene expression profile that seperates PD from controls (Schlachetzki et al., 2018). Yet, the systemic cytokine profile and the monocytic composition were not altered in our PD cohort.

2. Adaptive Immune System (Th-17/Treg axis)

More recently T-cells directed against alpha-synuclein, the protein aggregating in PD, linked PD pathology to the adaptive immune system (Sulzer et al., 2017). We assessed circulating T cells in PD and observed higher Th17 frequencies as well as elevated production of IL-17 by CD4+ T cells compared to controls (figure 1), and were able to show, that these PD derived Th-17 lympocytes are drivers of PD associated neurodegeneration (Sommer et al., 2018).

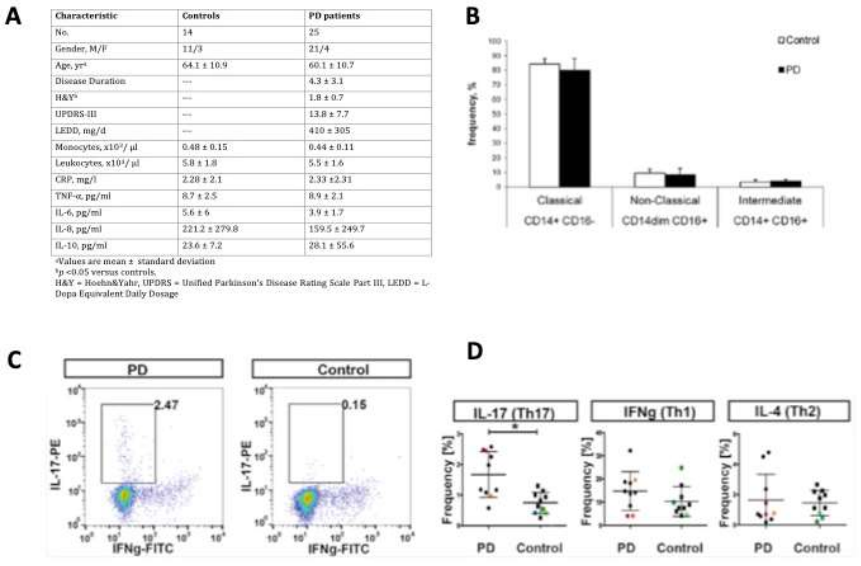
3. Gastrointestinal microbiome in PD

Increasing evidence suggests that altered gut microbiota may trigger or accelerate alpha-synuclein aggregation in the enteric nervous system in Parkinson's disease (PD). While several previous studies observed gut microbiome alterations, some studies observed changes in bacterial diversity indices, while others do not. Also, altered bacterial taxa itself show a considerable heterogeneity across studies.
Here, the composition of the gut microbiome in 71 PD patients and 30 healthy controls was analyzed, sequencing V3-V4 regions of the bacterial 16S ribosomal RNA gene in fecal samples. Our goal was (1) to evaluate whether gut microbiota are altered in

A

Characteristic	Controls	PD patients
No.	14	25
Gender, M/F	11/3	21/4
Age, yr[a]	64.1 ± 10.9	60.1 ± 10.7
Disease Duration	---	4.3 ± 3.1
H&Y[b]	---	1.8 ± 0.7
UPDRS-III	---	13.8 ± 7.7
LEDD, mg/d	---	410 ± 305
Monocytes, x10³/ µl	0.48 ± 0.15	0.44 ± 0.11
Leukocytes, x10³/ µl	5.8 ± 1.8	5.5 ± 1.6
CRP, mg/l	2.28 ± 2.1	2.33 ±2.31
TNF-α, pg/ml	8.7 ± 2.5	8.9 ± 2.1
IL-6, pg/ml	5.6 ± 6	3.9 ± 1.7
IL-8, pg/ml	221.2 ± 279.8	159.5 ± 249.7
IL-10, pg/ml	23.6 ± 7.2	28.1 ± 55.6

[a]Values are mean ± standard deviation
[b]p <0.05 versus controls.
H&Y = Hoehn&Yahr, UPDRS = Unified Parkinson's Disease Rating Scale Part III, LEDD = L-Dopa Equivalent Daily Dosage



The cytokine profile and the monocytic composition is not altered in PD (Schlachetzki et al., 2018) C-D) Analysis of circulating T cells of PD patients revealed increased frequencies of IL-17-producing CD4+ T cells (Th17 cells). Data are shown as means ± SD. *p < 0.05 (Sommer et al., 2018)

Dr. Marxreiter

a southern German PD cohort, (2) to delineate the influence of disease duration, and (3) to investigate the influence of PD associated covariates like constipation and coffee consumption. Aiming to control for a large variety of covariates, strict inclusion criteria were applied. Propensity score matching was performed to delineate the effect of remaining covariates (non-motor symptom (NMS) burden, constipation and coffee consumption) on microbiome composition.

Altered abundances of distinct bacterial classes, orders, families and genera were observed. Distinct bacteria like Sutterellaceae on family level were altered at early PD stages. On the other hand Enterobacteriaceae were increased and Lachnospiraceae reduced at advanced disease stages, only. Disease duration was significantly correlated with decreased abundancies of Lachnospiraceae, Clostridium XIVa, Faecalibacterium, F usicatenibacter, and Ruminococcus. After controlling for NMS burden, constipation and coffee consumption, only the genus Gemmiger remained significantly reduced in PD, and a reduced abundance of the genus Ruminococcus,, differed significantly between PD patients and controls.

Similar to previous studies, alterations of the taxa Lachnospiraceae, Ruminococcus, Faecalibacterium and Enterobacteriaceae were observed in PD. Yet, further controlling for PD associated covariates such as constipation and coffee consumption revealed a pivotal role of these covariates on microbiome composition in PD, highlighting the impact of these covariates on microbiome composition in PD, indicating that altered microbiota may indeed mediate the protective effect of i.e. coffee consumption and the negative effect of constipation in PD.

Contact:
Dr. Marxreiter
phone: +49 9131 85 46928
e-mail: franz.marxreiter@uk-erlangen.de

Invited lectures

Eröffnungssymposium Parkinson Fachklinik, 16.11.2019, Bad-Gögging, Darm und Hirn: vom modifizierten Mikrobiom zum Morbus Parkinson?

Symposium Therapeutic Strategies in Advanced Parkinson´s Disease with Country insights 29.11-30.11.2019 Vienna/Austria, Apomorphine injections in Parkinson's disease - Titration Protocols

Publications during funding period

Marxreiter F, Buttler U, Gassner H, Gandor F, Gladow T, Winkler J, et al. (2019) The Use of Digital Technology and Media in German Parkinson's Disease Patients. Journal of Parkinson's Disease, 29, 1–11

Marxreiter F, Utz K, Schlachetzki J C M, Seifert F, Schmidt M, Doerfler A, et al. (2019) Transient naming deficits associated with insular lesions in a patient with encephalitis. Neurocase, 00(00), 1–8

Marxreiter F, Gassner H, Borozdina O, Barth J, Kohl Z, Schlachetzki J. C. M, et al. (2018) Sensor-based gait analysis of individualized improvement during apomorphine titration in Parkinson's disease. Journal of Neurology, 20(2), 190

Sommer A, Marxreiter F, Krach F, Fadler T, Grosch J, Maroni M, Graef D, Eberhardt E, Riemenschneider M J, Yeo GW, Kohl Z, Xiang W, Gage FH, Winkler J, Prots I, Winner B. (2018) Th17 Lymphocytes Induce Neuronal Cell Death in a Human iPSC-Based Model of Parkinson's Disease. Cell Stem Cell. 23(1):123-131.e6

Minakaki G, Menges S, Kittel A, Emmanouilidou E, Schaeffner I, Barkovits K, Bergmann A, Rockenstein E, Adame A, Marxreiter F, Mollenhauer B, Galasko D, Buzás EI, Schlötzer-Schrehardt U, Marcus K, Xiang W, Lie DC, Vekrellis K, Masliah E, Winkler J, Klucken J. (2018) Autophagy inhibition promotes SNCA/alpha-synuclein release and transfer via extracellular vesicles with a hybrid autophagosome-exosome-like phenotype. Autophagy. 14(1):98-119

Gassner, H, Marxreiter F, Steib S, Kohl Z, Schlachetzki J C M, Adler W, et al. (2017) Gait and Cognition in Parkinson's Disease: Cognitive Impairment Is Inadequately Reflected by Gait Performance during Dual Task. Frontiers in Neurology, 8, 955–11. IF: 3.508

Marxreiter F, Winkler J, Uhl M, & Madžar D. (2017) A Case Report of Severe Delirium after Amantadine Withdrawal. Case Reports in Neurology, 9(1), 44–48

J56 - Final Report

01/01/2017 - 30/06/2019

Epigenetic reprogramming of macrophages

Dr. Katrin Palumbo-Zerr, Department of Medicine 3 – Rheumatology and Immunology

Clearance of apoptotic cells (ACs) is a key step during the resolution of inflammation and the maintenance of self-tolerance. In the current project we aim to dissect the immunometabolic consequences of the clearance of AC by macrophages. Uptake of ACs results in an anti-inflammatory response and reprogramming of macrophages. Our data suggest that these events are linked to a metabolic reprogramming and fundamental epigenetic changes in these cells.

Nuclear respiratory factor (NRF1) mediates the anti-inflammatory effects of AC engulfment

ChIPseq assay was performed to identify and analyze regulators mediating the ACs effects. It revealed several potential transcription factors to be differentially regulated by ACs phagocytosis. Among them NRF1 was the most promising candidate, as it has been described to interact with SIRT1 a Histone deacetylase, and also has been described to play a crucial role in mitochondrial biogenesis. NRF1 regulation was assessed using quantitative real time PCR. Phagocytosis of ACs significantly increased NRF1 mRNA levels on a basal level and short time stimulation with LPS.

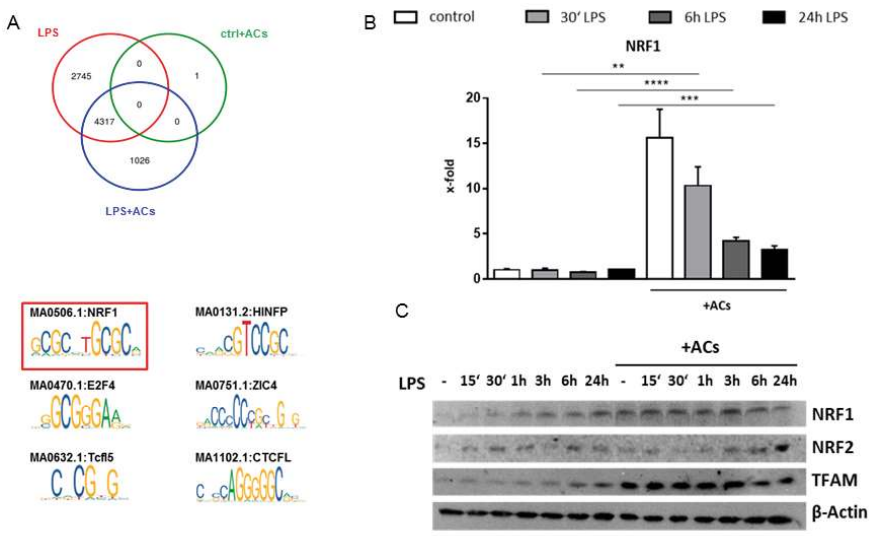
Western blot analysis demonstrated increased NRF1 protein expression levels upon LPS stimulation in the presence of ACs with constantly high protein levels up to 24h upon LPS stimulation. In contrast, hardly any changes in NRF2 expression, a closely related family member, were observed.

TFAM is a downstream target of NRF1 and therefore a suitable protein for measuring NRF1 activity. Similar to the NRF1 expression pattern, TFAM levels were drastically increased on basal level after AC ingestion, with protein levels.

mTOR is a upstream regulator of NRF1

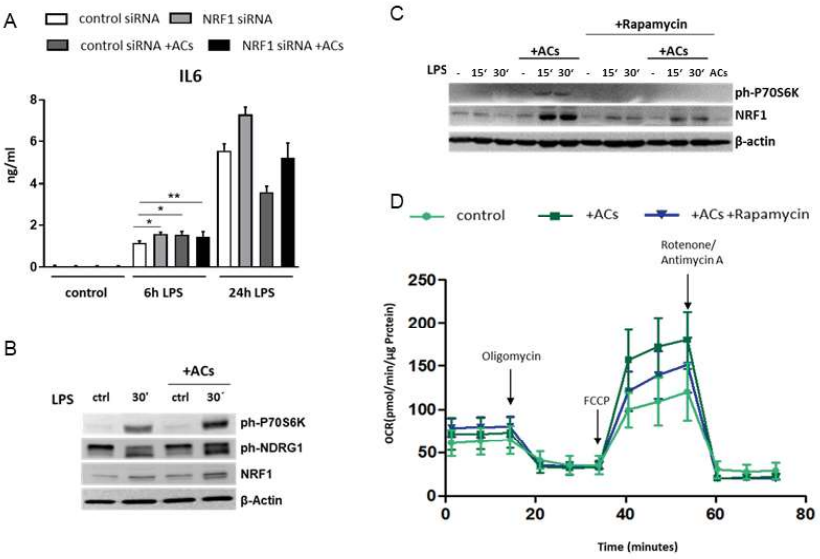
To investigate the role of NRF1 on AC engulfment, phagocytosis assays were performed. Transfection with NRF1 siRNA reversed the inhibitory anti-inflammatory effects of AC engulfment, with increased IL6 levels in cells transfected with NRF1 siRNA compared controls.

NRF1 is an important transcription factor for mitochondrial gene expression. Therefore, it is essential for mitochondrial biogenesis and cellular respiration.



(A) ChIPseq analysis of H3K27ac with enriched motifs from peak clusters; NRF1 motif marked red. (B) qPCR analysis of NRF1 mRNA. (C) Western blot analysis of NRF1, NRF2 and target gene TFAM.

Dr. Palumbo-Zerr



(A) ELISA-based analysis of IL6 cytokine release. (B) Western Blot analysis of mTOR downstream targets and NRF1. (C) Western Blot analysis of inhibitory effects of Rapamycin (mTORC1 inhibitor). (D) Seahorse analysis of oxygen consumption rate (OCR).

As mTOR is known to be a key regulator for metabolism, we presumed that mTOR might a possible upstream regulator of NRF1. Therefore we performed Western blot analysis to detect the activity of mTORC1, mTORC2 and NRF1.

Phosphorylation and thus activation of P70S6K, a downstream target of mTORC1, was increased by LPS stimulation and to a greater extent when additionally adding ACs. Whereas for phosphorylated NDRG1, a downstream target of mTORC2, was barely altered.

To further investigate a regulatory role of mTOR, we inhibited mTORC1 using Rapamycin is a potent inhibitor of mTORC1. Inhibition with Rapamycin inhibited the expression levels of NRF1, most prominently in macrophages stimulated with LPS and AC ingestion.

Moreover, we determined metabolic changes within the macrophage and detected a drastic increase in oxidative phosphorylation in macrophages that ingested ACs. Interestingly, these metabolic changes were linked to activation of mTOR singaling, as inhibition of mTOR by Rapamycin blocked the increase of oxygen consumption rate (OCR) in macrophages after uptake of ACs.

Future research will focus on the interconnection between the observed changes and the responsible molecular pathways to identify novel targets for the treatment of chronic inflammatory diseases.

Contact:
Dr. Palumbo-Zerr
phone: +49 9131 85 39379
e-mail: katrin.palumbo@uk-erlangen.de

Publications during funding period

none

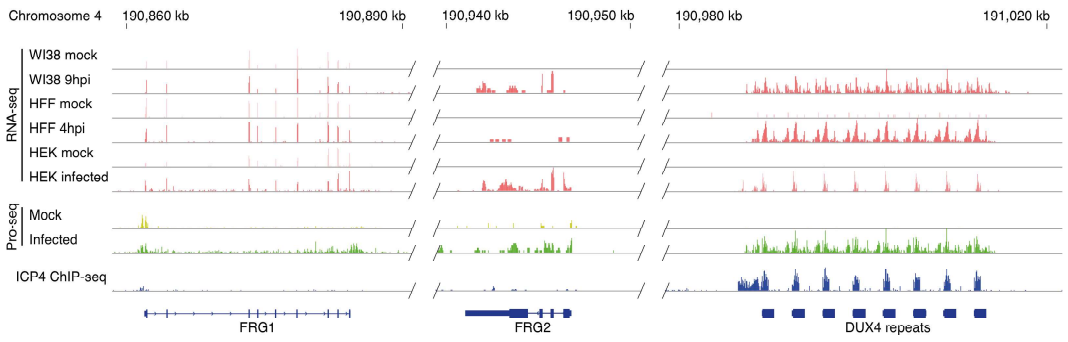
Herpesviruses and DUX4

Dr. Florian Full, Institute of Clinical and Molecular Virology

The embryonic transcription factor DUX4 causes Facioscapulohumeral Muscular Dystrophy (FSHD) and is the master regulator of zygotic genome activation (ZGA). We show that DUX4, hundreds of its target genes and germline specific endogenous retroviruses are actively induced by herpesviruses mimicking ZGA. Further experiments showed that depletion of DUX4 hampers viral gene expression, providing a rationale for targeting DUX4 as novel anti-herpesviral therapy.

Herpesviral infection is the cause of significant morbidity and mortality in humans worldwide, especially in immunocompromised individuals. Furthermore, two of the eight human herpesviruses, Kaposi's sarcoma-associated herpesvirus (KSHV) and Epstein-Barr virus (EBV), are classified as human carcinogens.

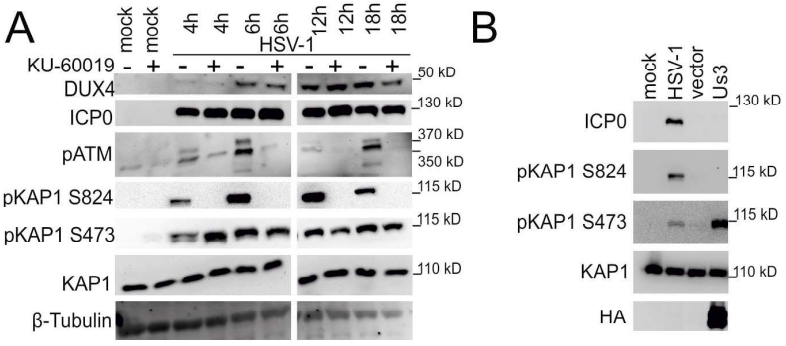
DUX4 is a transcription factor and regulator of zygotic genome activation (ZGA) during early embryogenesis. ZGA is crucial for maternal to zygotic transition at the 2-cell stage in order to overcome epigenetic silencing of genes and enable transcription from the zygotic genome. Moreover, aberrant expression of DUX4 in adult muscle cells is the cause of the genetic disorder Facioscapulohumeral Muscular Dystrophy (FSHD). Using RNA-Seq experiments we identified DUX4 as a transcription factor that is activated upon lytic replication of Herpes simplex virus (HSV), but not of adenoviruses, negative strand RNA viruses or positive strand RNA viruses. Further experiments also confirmed expression of DUX4 upon lytic replication of Human Cytomegalovirus (HCMV) and lytic reactivation of Kaposi's sarcoma associated herpesvirus (KSHV).



ICP4 ChIP-seq: Fox et al. 2017
Pro-seq: Birkenheuer et al. 2017
RNA-seq HEK: Full et al. 2018
RNA-seq HFF: Rutkowski et al. 2015
RNA-seq WI38: Wyler et al. 2017

RNA-Seq., Pro-Seq. and ICP-4-ChIP-Seq. tracks of FRG1 (DUX4-independent), FRG2 (DUX4-dependent) and the transcription factor DUX4 in uninfected vs. HSV-1 infected cells. DUX4 is induced upon HSV-1 infection by ICP-4 binding to the DUX4 locus.

Dr. Full



A: DUX4 expression by HSV-1 is dependent on phosphorylation of KAP-1 at serine 473 but independent of ATM. B: HSV-1 kinase Us3 phosphorylates KAP-1 at serine 473.

The DUX4 transcript observed upon herpesviral infection is identical to the DUX4 transcript found in ZGA. We demonstrate that DUX4 expression upon herpesviral replication leads to the induction of hundreds of DUX4 target genes, such as several members of the TRIM, PRAME and ZSCAN protein families. In addition, herpesviral infection results in DUX4-mediated transcription of long-terminal repeat (LTR) containing endogenous retrotransposons that are known to be activated during ZGA. Most ZGA genes and LTR-retrotransposons are exclusively expressed during early embryonic development; consequently almost no information about their function is available and their role during herpesviral infection remains elusive. Mechanistically we show that DUX4 expression is a direct consequence of herpesviral gene expression, as it can be stimulated by overexpression of herpesviral immediate early proteins and the viral kinase Us3. ChIP Seq experiments further show direct binding of herpesviral proteins to the DUX4 locus in the context of viral infection, indicating active induction of ZGA genes by herpesviruses. And finally we could demonstrate that depletion of DUX4 by CRISPR/CAS9 hampers viral gene expression and replication.

Taken together, our results show that infection with viruses from alpha-, beta- and gamma-herpesvirus subfamilies induces a DUX4-dependent germline-specific transcriptional program mimicking ZGA that is required for efficient viral replication. We hypothesize that herpesviruses exploit DUX4 function in order to overcome silencing of their genome and facilitate viral gene expression, similar to DUX4 function in ZGA. In addition, recent publications point an important role of DUX4 and DUX4 target genes in oncogenesis, which could contribute to development of KSHV- and EBV-associated cancer.

Contact:
Dr. Full
phone: +49 9131 85 26494
e-mail: florian.full@uk-erlangen.de

Invited lectures

Dr. Florian Full, Advances in Virology, Institut für Virologie, Uniklinikum Ulm, 05.06.2019: „Herpesviral infection induces germline-specific transcription mimicking zygotic genome activation“

Publications during funding period

Full F, van Gent M, Sparrer KMJ, Chiang C, Zurenski MA, Scherer M, Brockmeyer NH, Heinzerling L, Stürzl M, Korn K, Stamminger T, Ensser A, Gack MU (2019) Centrosomal protein TRIM43 restricts herpesvirus infection by regulating nuclear lamina integrity. Nature Microbiology 4(1): 164-176

J58 - Final Report

01/09/2016 - 28/02/2019

Oncology

Counteracting Wnt signaling

Dr. Dominic Bernkopf, Chair of Experimental Medicine II

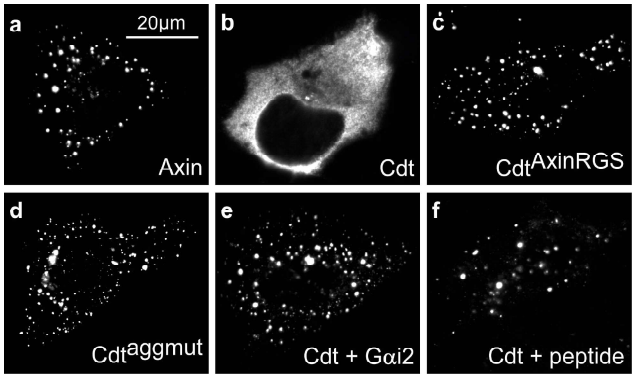
Wnt/β-catenin signaling is the major driving force of colorectal cancer making it an interesting therapeutic target. Our project focuses on conductin/axin2 a negative regulator of Wnt/β-catenin signaling sharing high homology with axin. Since axin-mediated β-catenin degradation correlates with axin polymerization and conductin does not polymerize, we hypothesize that induction of polymerization will enhance conductin-mediated β-catenin degradation, which could be exploited for cancer therapy.

An aggregon in the conductin RGS domain prevents polymerization

We could previously show by functional sequence comparison between axin and conductin through domain swapping that the axin regulator of G-protein signaling (RGS) domain is permissive for polymerization whereas the conductin RGS domain prevents polymerization. In silico analysis employing the TANGO algorithm identified an aggregon in the conductin RGS domain which is absent from axin. An aggregation-preventing point mutation triggered conductin polymerization, as seen by the formation of microscopically-visible spherical structures called “puncta”, suggesting that this newly identified aggregon blocks polymerization and accounts for the striking difference in distribution between axin (puncta) and conductin (diffuse).

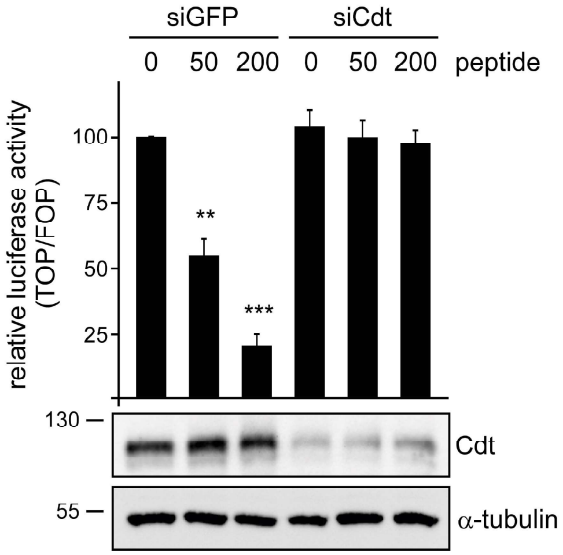
Conductin polymerization can be triggered by masking the aggregon

Importantly, conductin polymerization cannot only be induced by mutation of the aggregon. Also co-expression of Gα subunits triggered polymerization of conductin. The conductin RGS domain shows high homology to RGS domains in GTPase-activating proteins which bind to Gα subunits of trimeric G-proteins. Out of four tested Gα proteins (Gαo, Gαi1, Gαi2, Gαi3), Gαi2 showed the strongest induction of conductin polymerization and the strongest interaction with the conductin RGS domain in GST-pulldown assays suggesting that polymerization is induced by Gα binding. Consistently, induction of conductin polymerization could be decreased and increased via weakening (G184S Gαi2 point mutation) and strengthening (AlF4-treatment) the Gαi2-RGS interaction, respectively. Interestingly, Gαi2 binds the conductin RGS domain in close proximity to the identified aggregon, and we believe that the observed polymerization is triggered by masking the aggregon. In line with this, also co-expression of conductin fragments which contain the aggregon and can thereby interact with the RGS domain triggered polymerization of full length conductin. Strikingly, a small peptide containing the aggregon was sufficient to induce conductin polymerization, probably by masking the aggregon in conductin.



Induction of conductin polymerization. Fluorescence-based detection of axin (a), conductin (Cdt) (b), a Cdt mutant containing the axin RGS domain (c), a Cdt point mutant with inactive aggregon (d), Cdt co-expressed with Gαi2 (e) or the peptide (f).

Dr. Bernkopf



The peptide which induces conductin (Cdt) polymerization inhibited a reporter to measure the transcriptional activity of β-catenin (TOP/FOP) in colorectal cancer cells, and this inhibition was rescued by siRNA-mediated knockdown of conductin.

Triggering conductin polymerization inhibits Wnt/β-catenin signaling

Of note, triggering polymerization enhanced conductin-mediated β-catenin degradation and inhibition of β-catenin-dependent transcription in colorectal cancer cells. Enhanced inhibition of Wnt signaling was observed irrespective of how conductin polymerization was induced, i.e. aggregon mutation, co-expression of Gαi2 or the peptide. Finally, the polymerization-inducing peptide inhibited growth of colorectal cancer cells, most likely by activating endogenous conductin.

Our data reveal an aggregon in the conductin RGS domain which allows regulation of conductin polymerization and consequent inhibition of Wnt signaling. Physiologically, Gαi2 signaling might inhibit Wnt signaling via this mechanism. Therapeutically, the identified peptide holds potential for colorectal cancer treatment.

Contact:
Dr. Bernkopf
phone: +49 9131 85 29115
e-mail: dominic.bernkopf@fau.de

Invited lectures

Spring Meeting 2019 International Graduate School in Molecular Medicine Ulm, 12.04.2019, Ulm, Counteracting Wnt signaling in colorectal cancer through induction of conductin/axin2 polymerization

Publications during funding period

Bernkopf DB, Bruckner M, Hadjihannas MV and Behrens J (2019) An aggregon in conductin/axin2 regulates Wnt/beta-catenin signaling and holds potential for cancer therapy. Nat Commun. 10:4251

Bernkopf DB, Daum G, Bruckner M, and Behrens J (2018) Sulforaphane inhibits growth and blocks Wnt/beta-catenin signaling of colorectal cancer cells. Oncotarget 9:33982-33994

Bernkopf DB, and Behrens J (2018) Feedback regulation of mitochondrial homeostasis via Wnt/beta-catenin signaling. Mol Cell Oncol 5:e1458015

Bernkopf DB, Jalal K, Bruckner M, Knaup KX, Gentzel M, Schambony A, Behrens J (2018) Pgam5 released from damaged mitochondria induces mitochondrial biogenesis via Wnt signaling. J Cell Biol 217:1383-1394

Rauschenberger V*, Bernkopf DB*, Krenn S, Jalal K, Heller J, Behrens J, Gentzel M, and Schambony A (2017) The phosphatase Pgam5 antagonizes Wnt/beta-Catenin signaling in embryonic anterior-posterior axis patterning. Development 144:2234-2247
* shared first authorship

J59 - Final Report

01/07/2017 - 31/12/2019

Oncology

Immunotoxin induced anti-tumor immunity

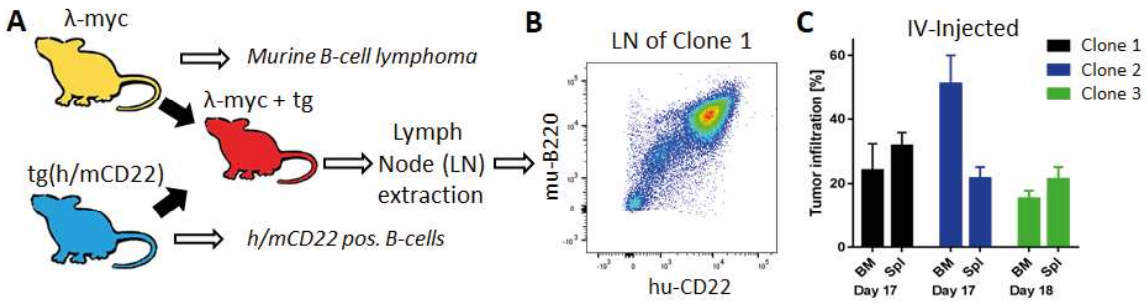
Dr. Fabian Müller, Department of Medicine 5 – Haematology and Oncology

The immunotoxin Moxetumomab pasudotox (or HA22) consists of a CD22 targeting antibody and Pseudomonas exotoxin. It specifically kills human B-cell lymphoma. Immunotoxins induce anti-tumor immune responses in patients with solid tumors. Hypothesizing that HA22 similarly modulates anti-lymphoma immunity, we aimed to establish an immune competent murine lymphoma model expressing human CD22 to then determine lymphoma infiltrating immune cells and changes within the tumor microenvironment induced by HA22.

The goal of this project was to establish an immune competent lymphoma mouse model to test effects of HA22 induced lymphoma cell death on the murine immune system. Because HA22 only binds human CD22, we generated a chimeric protein (h/mCD22) consisting of the intracellular domains of murine CD22 and the extracellular domains of human CD22. We hypothesized that HA22 binds the human parts on the cell surface and that the intracellular murine parts ensure correct transport of HA22 through the various intracellular compartments.

Using murine lymphoma cell line 291PC transduced with the h/mCD22 we confirmed that HA22 efficiently and specifically killed the h/mCD22 positive cells in a dose dependent manner supporting that the chimeric protein efficiently transports HA22 and thus, sensitizes the murine cells to a drug, which exclusively targets human CD22. However, the h/mCD22 positive 291PC failed to engraft stably in syngeneic mice.

Next, we crossbreed BL/6λ-myc mice which spontaneously develop aggressive B-cell lymphomas and BL6 mice which carry the h/mCD22 as transgene, thus, expressing h/mCD22 in every B cell. The resulting BL/6λ-myc / h/mCD22 mice spontaneously developed B-cell lymphoma highly expressing the h/mCD22. Three h/mCD22 positive lymphoma clones from distinct mice were extracted from lymph nodes and serially transplanted into recipient BL/6h/mCD22 mice. Next, we characterized lymphoma growth rates in vivo, and characterized lymphoma infiltrating immune cells of the three primary murine lymphoma models at base line. Distinct from human myc-driven lymphoma, subcutaneously growing tumors were infiltrated by T-cells or myeloid cells by less than 0.5% and by less than 1%, respectively. When lymphoma cells were injected systemically into the tail vein, tumor cells grew in bone marrow, spleen, and lymph nodes and the lymphoma showed

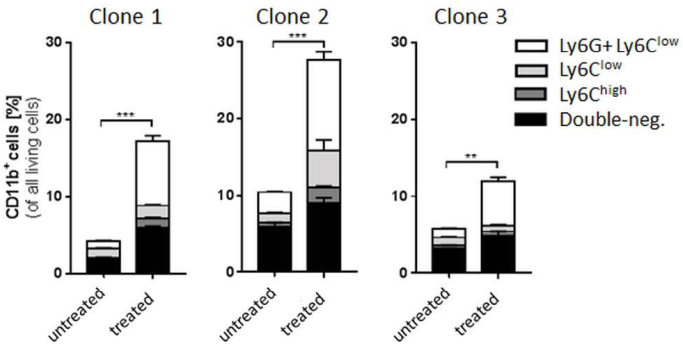


(A) Breeding scheme to generate myc-driven, h/mCD22 positive primary murine lymphomas isolated from lymph node extracts (B). When serially transplanted by intravenous injection, lymphoma grew systemically in bone marrow (BM) and spleen (spl).

Dr. Müller

substantial immune cell infiltration including myeloid, T-, and innate cells. As such, the systemic but not the subcutaneous model resembled immune infiltration found in the human lymphoma counterpart.

As in humans, myeloid cell infiltration dominated. We treated lymphoma bearing mice with doxorubicin, a known activator of lymphoma infiltrating, suppressive myeloid cells which led to substantial increase of myeloid cells. These new, tumor-infiltrating myeloid cells were predominantly of granulocytic phenotype. Treatment with immunotoxin HA22 was optimized by increasing dosing frequency. Maintaining high blood levels over time reduced tumor burden by up to 50-fold in BM and 20-fold in spleen. Analyses of effects of HA22 on the murine immune system including phenotypic and functional characterization of the lymphoma invading immune cells are ongoing.



Treating mice bearing either of the three isolated lymphoma clones led to a substantial increase in CD11b pos myeloid cells which were dominated by Ly6G positive granulocytes.

Contact:
Dr. Müller
phone: +49 9131 85 39542
e-mail: fabian.mueller@uk-erlangen.de

Patents/ Licenses during funding period

WO 2019121687

Publications during funding period

Müller F, Cunningham T, Beers R, Bera TK, Wayne AS, Pastan I (2018) Domain II of Pseudomonas Exotoxin Is Critical for Efficacy of Bolus Doses in a Xenograft Model of Acute Lymphoblastic Leukemia. Toxins, 10(5), 210

Müller F, Cunningham T, Stookey s, Tai C-H, Burkett S, Jailwala P, Stetler Stevenson M, Cam MC, Wayne AS, Pastan I (2018) 5-Azacytidine prevents relapse and produces long-term complete remissions in leukemia xenografts treated with Moxetumomab pasudotox. Proceedings of the National Academy of Sciences 115 (8) E1867-E1875

J61 - Final Report

01/01/2017 - 30/06/2019

Others

Extending joint models in biomedical outcomes

Dr. Elisabeth Waldmann, Department of Medical Informatics, Biometry and Epidemiology

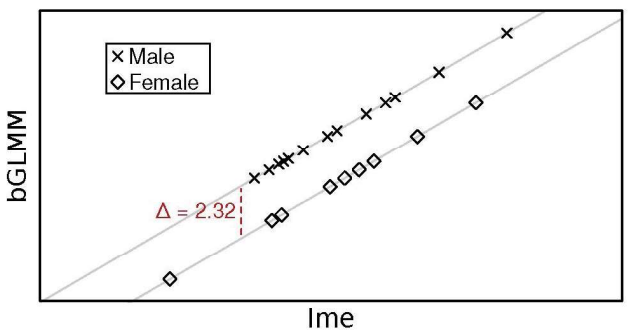
Biomedical studies often aim at two goals: prediction the development over time, or risks of events. I.e. variables of interest are collected repeatedly or data on times of events is reported. In many cases those two outcomes are related and collected alongside each other, analysis however is done separately. Models connecting the two structures are called joint models. This project aims at extending them in terms of variable selection and beyond the mean modelling.

Variable Allocation via Boosting

The popularity of joint models for longitudinal and time-to-event-data has grown rapidly in the last few decades. Those models are based on three sub predictor functions which either only have an impact on one of the outcomes or on both simultaneously. Gradient boosting is a statistical learning method which has the inherent ability to select and estimate variables at the same time. At the beginning of this project, we implemented the first boosting algorithm and hence the first variable selection tool for joint models. This part of the project aims at allocating the variables automatically to parts of the model: Since researchers do not necessarily know beforehand which covariate has an influence on which part of the outcome, the here suggested algorithm is able to do both - select and allocate variables to the correct sub-predictor. The algorithm that fulfils this task is based on our original algorithm from 2017 with changes towards non-cyclic updating scheme and adaptive step lengths. It was tested via simulation studies and is now applied to data sets from different medical studies.

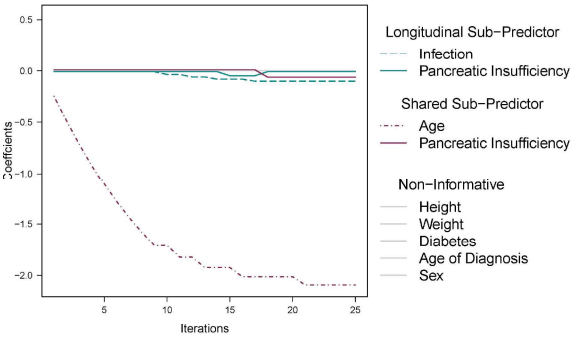
Improving likelihood-based boosting for mixed effects models

While extending likelihood-based boosting techniques to a complete analysis of both the longitudinal and the survival submodel of a joint model, a severe malfunction of current likelihood boosting approaches for linear mixed models had been discovered. In the case of longitudinal data containing time-invariant covariates like e.g. gender or treatment group, current algorithms tend to compensate effects actually evolving from the covariates by falsely assigning different random intercepts to each individual and thus leaving the true fixed effects at zero. This problem occurred in simulation as well as in real data and could be solved with different major and minor improvements of the existing method.



A maximum likelihood approach yields the fixed effect estimate of 2.32 for the time-invariant covariate ‚gender' while the estimate by a likelihood-boosting method remains zero, but with the random intercept of each female subject lowered by 2.32.

Dr. Waldmann



Coefficient paths for the allocation algorithm show that some candidate variables /are relevant /for both sub predictors, but they can also change from the longitudinal to the joint sub predictor (note: but not the other way around).

Apart from reweighting the different step lengths for fixed and random effects updates, these improvements included undocking the random effects update from the fixed effects selection process in order to obtain a fair comparison as well as correcting the random effects update in each iteration. This ensures that the random effects are uncorrelated with all time-invariant covariates. The updated algorithm not only solved the issue of random effects growing too quickly, but also reduced the computational effort by a crucial amount making the technique much more attractive for application.

Adaptive Step Lengths for more balanced models

A further more general problem that came to our attention in the course of the project was the issue of step length in gradient boosting algorithms for more than just one outcome. This well-known issue was tackled in a master's thesis and numerical as well as analytical solutions were found for the context of generalized additive models for location shape and scale. The extension of those results to joint models is one of the future plans of the group.

Contact:
Dr. Waldmann
phone: +49 9131 85 22722
e-mail: elisabeth.waldmann@fau.de

Publications during funding period

Gayawan E, Adebayo S B, Waldmann E (2019) Modelling the spatial variability in the spread and correlation of childhood malnutrition in Nigeria. Statistics in Medicine. doi: 10.1002/sim.8077

Waldmann E (2018) Quantile Regression: A short story on how and why. Biometrical Journal. doi:10.1002/bimj.20160015

Waldmann E, Taylor-Robinson D, Klein N, Kneib T, Pressler T, Schmid M, Mayr A (2017) Boosting Joint Models for Longitudinal and Time-to-Event Data. Biometrical Journal. doi:10.1002/bimj.20160015

Mayr A, Hofner B, Waldmann E, Hepp T, Meyer S, Gefeller O (2017) An update on statistical boosting in biomedicine. Computational and Mathematical Methods in Medicine. vol. 2017, Article ID 6083072, doi:10.1155/2017/6083072

Gefeller O, Hofner B, Mayr A, Waldmann E (2017) Predictive Modelling Based on Statistical Learning in Biomedicine, Computational and Mathematical Methods in Medicine., Article ID 4041736, doi:10.1155/2017/4041736

J62 - Progress Report

01/08/2017 - 31/01/2020

Mechanisms of neutrophil infiltration in rheumatoid arthritis

Dr. Anika Grüneboom, Department of Medicine 3 – Rheumatology and Immunology

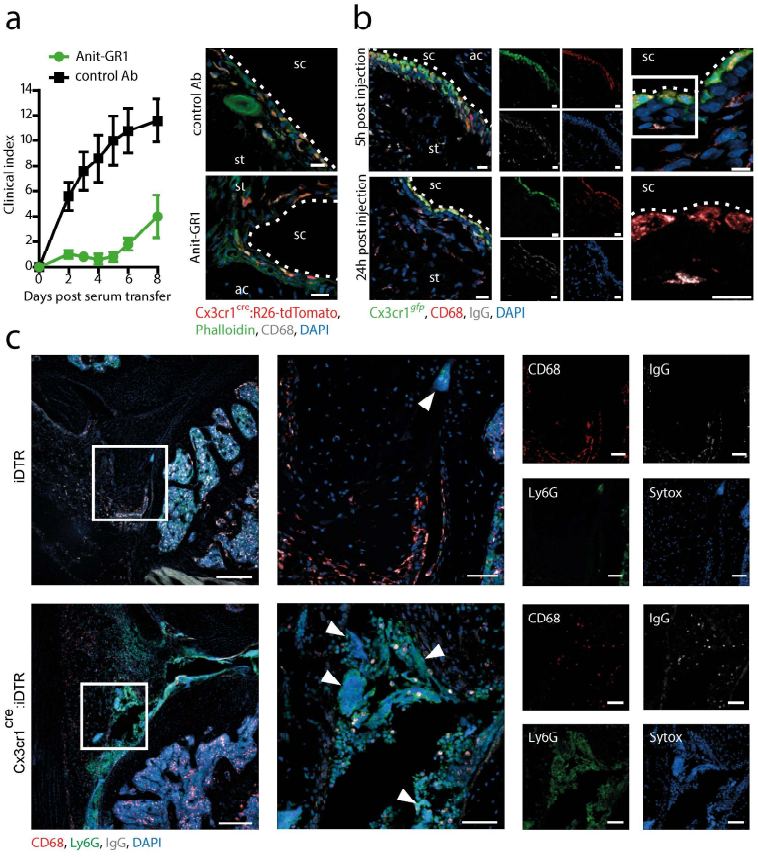
Neutrophil play a key role during acute infections and tissue injury-induced inflammation as they are the first leukocytes recruited to inflammatory sites. Neutrophils are also highly abundant in the inflamed joints of rheumatoid arthritis (RA) patients, but their exact contribution to initiation, propagation and resolution of this autoimmune disease is poorly understood. This project focusses on the role of neutrophils in the onset of RA and their interaction with tissue resident macrophages.

Neutrophil granulocytes display a bi-phasic recruitment into inflamed joints

The healthy synovial joint tissue is an immune privileged site that lacks immune cell infiltration. In contrast the K/BxN serum transfer arthritis (STA) induces a massive recruitment of immune cells, mainly neutrophil granulocytes, into the synovial tissue and the synovial cavity. Instead of a homogenous infiltration we observed an accumulation of neutrophils in the synovial cavity at the very early onset of arthritis. Only at the peak of inflammation neutrophils were additionally located in the synovial tissue. This indicates a bi-phasic recruitment of neutrophil granulocytes with the synovial cavity as their preferred target tissue.

Synovial lining integrity is not disintegrated by invading neutrophils but immune complexes

In line with our infiltration studies we identified a membrane-like structure formed by CX3CR1+ macrophages (MΦs), which secludes the intra-articular space from the exterior synovial tissue. These joint lining MΦs undergo massive morphological changes during the onset of STA. They abrogate their cell-cell contacts, acquire a palisade like shape, and phagocytose the infiltrating neutrophils. To cla-



Neutrophils in STA: a) Neutrophil depletion results in a reduced clinical index but continuance of lining disruption. b) Lining disruption is mediated by early IgG uptake of lining macrophages. Scalebars = 10µm. c) Depletion of lining macrophages results in increased neutrophil infiltration. Scalebars = 100 µm.

Dr. Grüneboom

rify if the invading immune cells induce the disintegration of the MΦ lining we depleted neutrophils and inflammatory monocytes, which resulted in an alleviated clinical STA outcome. However, histological analysis of the MΦ morphology did not show any effects on the membrane integrity. Thus, we injected labelled immune complexes to study their influx kinetics. While neutrophils were found in the synovial cavity about 24 hours after STA induction the labelled IgG was already detectable 5 hours after injection. The immune complexes were ingested by both synovial tissue MΦs and lining MΦs. Based on these findings we conclude an activation and disintegration of the synovial membrane by immune complexes independent of invading inflammatory immune cells.

Synovial lining macrophages are suited as therapeutic targets in RA

To further investigate the role of the lining MΦs we used Cx3cr1creiDTR mice where CX3CR1+ cells can be depleted by systemic administration of diphtheria toxin (DT). The DT-mediated MΦ depletion resulted in a massively accelerated infiltration of neutrophils into the synovial cavity. This hints to an important immune-regulatory function of the synovial lining MΦs by maintaining a physical barrier that secludes and protects the intra-articular structures. Based on single-cell sequencing of the CX3CR1+ lining MΦs, we identified features that are otherwise typical of barrier-forming epithelial cells. According to this we tested the tyrosine kinase inhibitor Imatinib, which stabilizes endothelial cell-cell contacts. The administration of Imatinib resulted in an alleviated clinical outcome of STA turning the lining MΦs into a novel target structure for therapeutic treatment of RA.

Contact:
Dr. Grüneboom
phone: + 49 9131 85 43216
e-mail: anika.grueneboom@uk-erlangen.de

Invited lectures

ECTS PhD Training, 07.-10. September 2019, Bologna, Italy (A. Grüneboom): “A network of trans-cortical capillaries as mainstay for blood circulation in long bones”

European Congress of Rheumatology, 12.-15. June 2019, Madrid, Spain (A.Grüneboom, M. Gunzer): “LSFM and catching up in knee joint imaging”

Guest Lecture - SFB1116, 05. June 2019, Düsseldorf, Germany (A. Grüneboom): “Mesoscopic and microscopic imaging of immuno-vascular processes”

Awards

Avrion Mitchison Price 2019 - DRFZ, Stephan Culemann and Anika Grüneboom, 03. November 2019, Berlin

Publications during funding period

Culemann S*, Grüneboom A*, Nicolás-Ávila J A, Lämmle K, Eberhardt M, Ferrazzi F, Schicht M, Weidner D, Fischer K, Gelse K, Faas M, Pfeifle R, Rothe T, Renner N, Haseloff R F, Ekici A, Bäuerle T, Blasig I E, Vera J, Schett G, Hidalgo A, Krönke G. (2019). Spatiotemporal molecular profiling of synovial macrophages reveals a locally renewing barrier of membrane-forming macrophages shielding the joint. Nature, 572:670–67 *equal contribution

J63 - Progress Report

01/12/2017 - 31/05/2020

IL-3 in inflammatory bowel disease

Dr. Sebastian Zundler, Department of Medicine 1 – Gastroenterology, Pneumology and Endocrinology

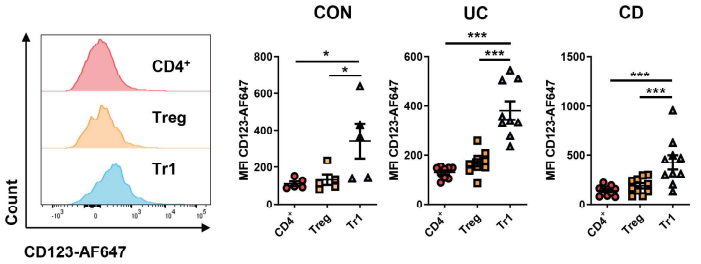
In this project, we seek to elucidate the role of interleukin-3 (IL-3) in the pathogenesis of inflammatory bowel diseases (IBD). The data generated during the last year further support a protective role of IL-3 in experimental colitis, while it seems that this is associated with altered function of regulatory T cell subsets. Single-cell and bulk RNA sequencing data as well as new genetic mouse models will we used to gain further insights.

Inflammatory bowel diseases (IBD) such as ulcerative colitis (UC) and Crohn's disease (CD) arise from a complex interplay of environmental and host factors, eventually resulting in undercontrolled activation of the intestinal immune system. Multiple cytokines have been shown to play a key role in this process, but the contribution of IL-3 is so far unclear. Thus, our goal is to explore the function of IL-3 in patient samples and in experimental in vivo models.

Having previously shown that IL-3 mRNA is upregulated in the lamina propria of patients with UC compared with patients with CD and control patients, we could now show with immunohistochemistry that this is related to an increase in IL-3-expressing CD4+ T cells. Moreover, in a large cohort of IBD patients, high expression of IL-3 mRNA was associated with short flare-free survival. The IL-3 receptor CD123 was expressed in substantially higher levels on Tr1 compared with Tregs and total CD4+ T cells. Seen in the context of the rest of our data, this argues for unsuccessful attempts to activate an IL-3-dependent regulatory pathway in the setting of severe ulcerative colitis.

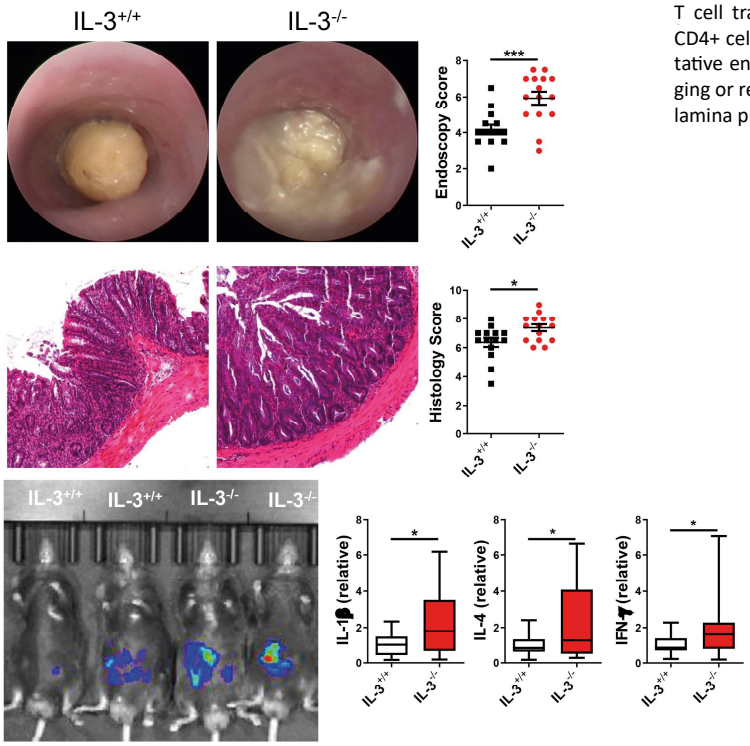
Using naïve CD4+ T cells from Il3-/- and Il3+/+ mice, we performed transfer colitis in Rag1-/- mice. In line with previous data in the oxazolone colitis model, we observed a severer disease course in mice that received Il3-/- cells than in mice that received Il3+/+ cells. Since earlier data had suggested that IL-10-expressing Tr1 cells might be a target of IL-3, we crossed Il3-/- mice to reporter mice for IL-10 and Foxp3. In preliminary analyses, we observed that unlike in Il3+/+ control animals, Il10-expressing CD4+ T cells in the intestinal lamina propria of Il3-/- mice co-express Foxp3, further substantiating the view that Il3 modulates the differentiation of regulatory T cell subsets such as the balance between Tregs and Tr1 cells. Freshly acquired bulk RNA-sequencing data from T cell transfer colitis lamina propria cells and single cell RNA-sequencing data of CD3+CD123+ T cells in the inflamed gut, will now further shape our knowledge on the mechanisms and targets of IL-3.

Another valuable tool on this way will be newly generated Il3r-/- mice, which will serve as donors of regulatory T cell subsets for co-transfer experiments in Rag1-/- mice. Moreover, we now dispose of conditional Il3r knockout mice, which we have almost completely crossed to CD4-Cre mice and can be used to corroborate earlier results.



IL3 receptor (CD123) expression by T cells. Representative and quantitative flow cytometry of CD123 expression on total CD4+ T cells, Tregs and Tr1 cells in healthy donors (CON), Crohn's disease (CD) and ulcerative colitis (UC).

Dr. Zundler



T cell transfer colitis after transfer of Il3+/+ and Il3-/- CD4+ cells into Rag1-/- mice. Representative and quantitative endoscopy and histology, representative IVIS imaging or reactive oxygen species and cytokine secretion by lamina propria cells.

Taken together, our so far data suggest that IL-3 promotes protective pathways effectuated by regulatory Tr1 cells in mucosal immunity. However, increased activation of this pathway in UC seems to be futile. Further deciphering the mechanisms of IL-3 and exploring the reasons for insufficiency of IL-3-driven counter-regulation in active IBD will be the key tasks for the remaining project period.

Contact:
Dr. Zundler
phone: + 49 9131 85 45022
e-mail: sebastian.zundler@uk-erlangen.de

Awards

"Karriereförderpreis" of the "Ernst Jung-Stiftung für Wissenschaft und Forschung", Sebastian Zundler, 23.05.2019, Hamburg

"Zukunftspreis" of the "Deutsche Arbeitsgemeinschaft Chronisch entzündliche Darmerkrankungen (DACED)", Sebastian Zundler, 14.06.2019, Mainz

"Innovationspreis" of the "Medizinischer Fakultätentag Deutschland", Sebastian Zundler, 26.09.2019, Berlin

"Martin Gülzow-Preis" of the "Deutsche Gesellschaft für Verdauungs- und Stoffwechselerkrankungen", Sebastian Zundler, 03.10.2019, Wiesbaden

Publications during funding period

Zundler S, Becker E, Schulze LL, Neurath MF (2019) Immune cell trafficking and retention in inflammatory bowel disease: mechanistic insights and therapeutic advances. 68(9):1688–700

Zundler S, Becker E, Spocinska M, Slawik M, Parga-Vidal L, Stark R, et al. (2019) Hobit- and Blimp-1-driven CD4+ tissue-resident memory T cells control chronic intestinal inflammation. Nat Immunol. 20(3):288–300

Lichnog C, Klabunde S, Becker E, Fuh F, Tripal P, Atreya R, […] and Zundler S (2019) Cellular Mechanisms of Etrolizumab Treatment in Inflammatory Bowel Disease. Front Pharmacol. 10:39

J64 - Progress Report

01/10/2017 - 31/08/2020

Renal and Vascular Research

Nephroprotection by HIF-hydroxylase inhibitors

Dr. Steffen Grampp, Department of Internal Medicine 4 – Nephrology and Hypertension

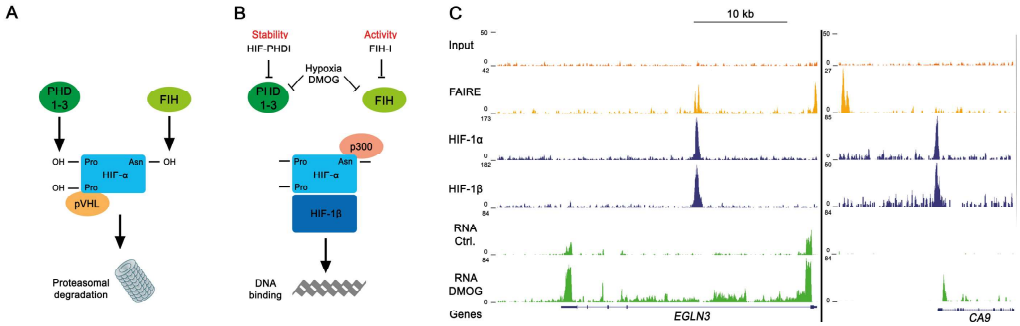
In Acute kidney injury (AKI) restricted blood flow and oxygen supply lead to tissue hypoxia and ultimately to cell death. There is a great body of evidence from rodent models of AKI that the pre-conditional stabilization of hypoxia-inducible factors (HIFs) in renal tubular epithelial cells leads to an improved kidney function. However, the underlying mechanisms of the HIF response and regulation in AKI are poorly understood, and it is unclear whether these effects can be translated into human disease.

Background

HIFs are transcription factors regulated by prolyl-hydroxylases (PHD) and factor inhibiting HIF (FIH1) with PHDs regulating the HIF-stability and FIH regulates the HIF-activity. New PHD inhibitors (HIF-PHDI) have been developed for the treatment of chronic anemia in patients with chronic kidney disease. Through stabilization of HIF-2α they induce the HIF target gene erythropoietin (EPO) kidney cells. However, the new generation of PHD-inhibitors does not only stabilize HIF-2α and induce EPO expression specifically in the kidney, but potentially lead to the induction of HIF in most of the cells of the organism mimicking a state of acute hypoxia with unselective target gene induction. Furthermore these compounds only inhibit the PHD axis of HIF regulating enzymes, but not FIH1. A potential additional protective HIF-effect by FIH1 inhibition e.g. in the setting of AKI has not been tested. This work will test both approaches (PHD and FIH1 inhibition) in human renal tubular cells and AKI animal models which appear to be most relevant for a potential short time use of HIF-PHDIs in the setting of AKI.

FIH1 inhibition increases the hypoxic gene induction in vitro and in vivo

The new generation of selective PHD inhibitors showed a reduced induction of HIF target genes in comparison to the pan-hydroxylase inhibitor dimethyloxalylglycine (DMOG). Pharmacobiological studies revealed that these compounds only inhibit PHD enzymes. In order to address the relevance of an additional FIH-inhibition for HIF target gene induction we used a specific FIH1-inhibitor DM-NOFD. The results showed an increased induction of HIF target genes in hPTEC when both HIF regulation enzymes, PHD and FIH were inhibited. In vivo studies using C57BL/6 WT mice also showed an increased HIF target gene induction when both HIF regulating enzymes were inhibited.



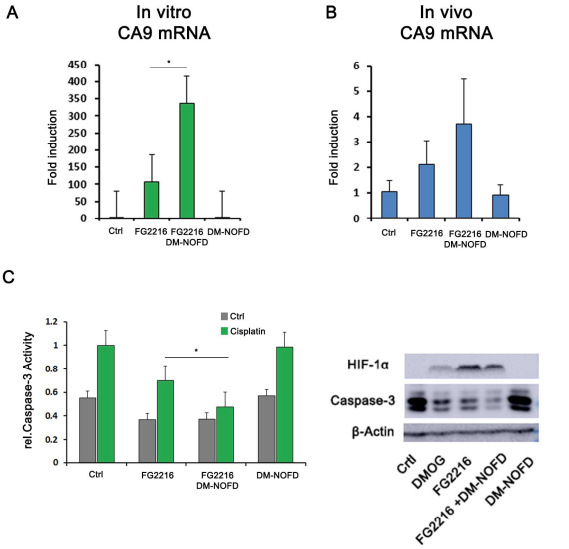
Schematic overview of the HIF-Pathway in A normoxic and B hypoxic conditions. C FAIRE-, HIF-ChIP- and RNA-seq tracks reveal robust HIF-binding to the EGLN3 and CA9 gene loci and increased expression of both genes.

Dr. Grampp

Preconditional HIF-Stabilization protects hPETC in an in vitro AKI model

Preconditional HIF-stabilization has a nephroprotective effect in rodent models of AKI. To explore this effect in human PETC, we used the in vitro cisplatin AKI model. Results of Caspase-3-Apoptose assays indicated a cellprotective effect when HIF is stabilized prior to cisplatin incubation. Additional inhibition of FIH1 displayed the strongest reduction of apoptosis in hPETC. Taken together these experiments indicate a promising nephroprotective potential of combined PDH and FIH1 inhibition.

Further experiments will focus on the underlying molecular biological mechanisms and candidate pathways as well as if these effects can be translated into in vivo AKI models.



A/B CA9 mRNA expression in hPTC and C57Bl/6 mice. Increased target gene induction was observed with combined PHD and FIH1 inhibition. C Caspase-3 assay of cisplatin in vitro AKI model revealed a cell-protective effect after combined PHD and FIH inhibition

Contact:
Dr. Grampp
phone: + 49 9131 85 40112
e-mail: steffen.grampp@uk-erlangen.de

Publications during funding period

Lauer V, Grampp S, Platt J, Lafleur V, Lombardi O, Choudhry H, Kranz F, Hartmann A, Wullich B, Yamamoto A, Coleman ML, Ratcliffe PJ, Mole DR, Schödel J (2019) Hypoxia drives glucose transporter 3 expression through HIF-mediated induction of the long non-coding RNA NICI. J Biol Chem. pii: jbc.RA119.009827. doi:10.1074/jbc.RA119.009827. [Epub ahead of print]

J65 - Progress Report

01/11/2017 - 30/04/2020

Renal and Vascular Research

T-System Regulation by Glucocorticoids

Dr. Thomas Seidel, Institute of Cellular and Molecular Physiology

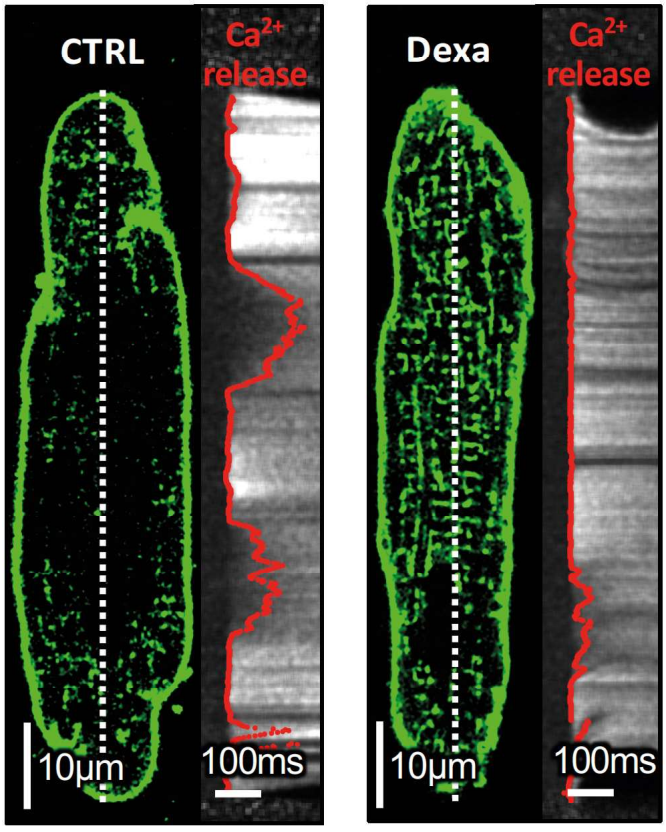
The transverse tubular system (t-system), a specialized system of membrane invaginations in cardiac myocytes, facilitates cardiac excitation-contraction coupling. In heart failure, the t-system undergoes severe remodeling, which impairs cardiac contraction, adds to heart failure progression and prevents recovery. In this project we investigate mechanisms underlying t-system remodeling in heart failure with the ultimate goal to identify strategies for preventing and reversing heart failure.

Glucocorticoids preserve the t-system and excitation-contraction coupling in cardiomyocytes

Culturing isolated cardiomyocytes is a common model of t-system loss and remodeling. We discovered that dexamethasone and corticosterone prevent t-system remodeling in cell culture. By application of spironolactone and mifepristone in combination with dexamethasone, we identified the glucocorticoid receptor (GR) as the major mediator of these effects. Moreover, co-immunostaining of L-type calcium channels (LCC) and ryanodine receptors (RyR) revealed improved spatial coupling between LCC and RyR clusters in dexamethasone-treated cells, a prerequisite for cardiac excitation-contraction coupling. Fast line scan imaging with a confocal microscope of living cardiomyocytes loaded with a calcium indicator showed that the synchrony of intracellular calcium release was significantly higher in dexamethasone-treated cells than in control cells. Thus, dexamethasone enhanced excitation-contraction coupling.

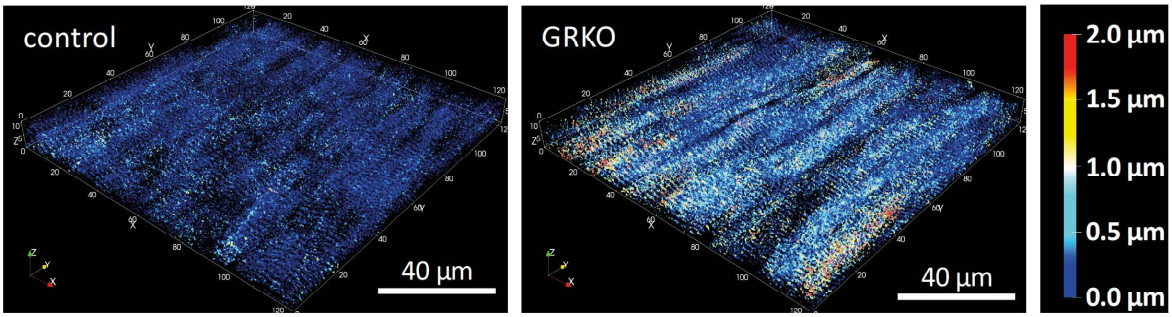
Junctional coupling is decreased in glucocorticoid receptor knockout mice

To assess if glucocorticoid receptor signaling is important for t-system maintenance in vivo, we investigated hearts obtained from cardiac-specific glucocorticoid receptor knockout (GRKO) mice and compared them to control hearts. GRKO increased RyR-LCC distances, indicating t-system remodeling and impaired junctional integrity. These results were confirmed by 3D STED microscopy and suggest that GR signaling is important for t-system structure also in vivo, which fits to the finding that GRKO mice develop signs of heart failure and reduced cardiac contractility.



T-System with surface membrane (green) and Ca2+ release times (red) determined by line scans (dotted line) in adult rat cardiomyocytes treated with either vehicle (CTRL) or 1 µM dexamethasone (Dexa). Dexa improves t-system and Ca2+ release synchrony.

Dr. Seidel



Color map of distances between ryanodine receptors and L-type Ca2+ channels in cardiac tissue from control or cardiac glucocorticoid receptor knockout (GRKO) mice. Increased distances in GRKO indicate impaired junctional coupling and t-system loss.

GR effect on the t-system does not involve upregulation of BIN1, JPH2 or Cav3

We then asked if maintenance of the t-system is associated with higher expressions of the t-system associated proteins BIN1, Cav3 and JPH2. However, using qPCR and Western blotting, we did not find any significant increases in mRNA or protein levels of these proteins in dexamethasone-treated cells, suggesting t-system preservation by glucocorticoids is not mediated by direct regulation of BIN1, JPH2 or Cav3.

Glucocorticoids preserve the t-system by upregulation of autophagic flux

Autophagy is a highly preserved cellular mechanism upregulated in situations of stress and nutrient deprivation to maintain cellular energy homeostasis. Furthermore, it is involved not only in the degradation of dysfunctional proteins and organelles, but also in the recycling of membrane structures. Dexamethasone strikingly upregulated autophagy and autophagy-related genes in cardiomyocytes. Moreover, autophagy blockers accelerated t-system loss and blunted the effects of dexamethasone, while autophagy enhancers, such as rapamycin, slowed down t-system loss. From this we conclude that autophagy is crucial for the maintenance of the cardiomyocyte t-system and that glucocorticoids preserve the t-system through upregulation of autophagic flux.

Contact:
Dr. Seidel
phone: + 49 9131 85 22303
e-mail: thomas.seidel@fau.de

Invited lectures

Seminar Series at the Institute for Experimental Cardiovascular Medicine, University of Freiburg, Germany, October 8, 2019, Freiburg, “The transverse tubular system: A diagnostic, prognostic and therapeutic target in heart failure?”

Awards

Poster award at the Meeting of the German Physiological Society (DPG) 2019, Ulm. “Structural and functional assessment of human ventricular myocardium: T-system density correlates with contractile parameters of myocardial slices from end-stage heart failure patients.” M. Abu-Khousa, D. Fiegle, A. Dendorfer, T. Volk, T. Seidel (laureate: Maha Abu-Khousa, date: October 2, 2019)

Publications during funding period

Seidel T, Fiegle D J, Baur T J , Ritzer A, Nay S, Heim C, Weyand M, Milting H, Oakley R H, Cidlowski J A, Volk T (2019) Glucocorticoids preserve the t-tubular system in ventricular cardiomyocytes by upregulation of autophagic flux. Basic Res. Cardiol. 114(6):47 doi 10.1007/s00395-019-0758-6

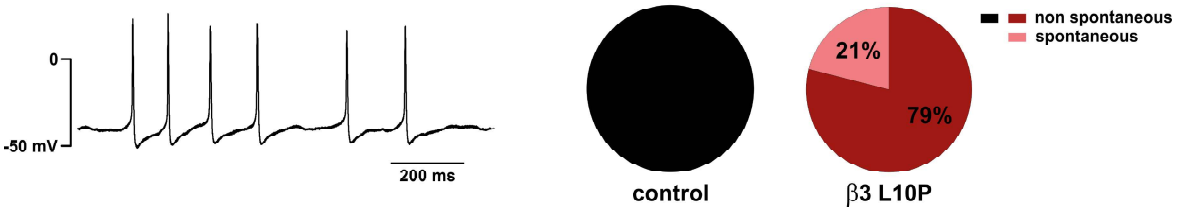
β subunits: adding pieces to the puzzle of pain

Dr. Esther Eberhardt, Department of Anaesthesiology

Chronic pain is a common health problem for which therapy remains often unsatisfactory. Rare mutations in voltage-gated sodium channels (Navs) have helped our understanding of the pathophysiology of pain. The aim of this study is to use human induced pluripotent stem cells from patients with rare variants in Nav accessory proteins to elucidate the contribution of these β subunits to cellular excitability and to obtain insights in more complex polygenetic pathomechanisms of pain.

Voltage-gated sodium channels are important for generation and propagation of action potentials in excitable cells. Their expression and gating is further modulated by regulatory proteins (β subunits). Mutations of these accessory subunits have been linked to arrhythmias and epilepsy syndromes and have recently also been found in patients suffering from the chronic pain syndrome erythromelalgia.

We obtained skin biopsies of two erythromelalgia patients carrying mutations in β1 (p.R125C) and β3 (p.L10P) respectively which both show increased spontaneous activity of their C-fibers in microneurography recordings indicating a pathology in small sensory neurons. Using a fibroblast reprogramming approach, we successfully generated human induced pluripotent stem cells (hiPSCs) from both patients and two age-matched healthy controls which we differentiated into patient-derived nociceptors. The electrophysiological properties of these sensory neurons were further analysed with the patch-clamp technique and multi electrode array (MEA) recordings to elucidate the impact of the mutations on neuronal excitability after a maturation period of up to 77 days.

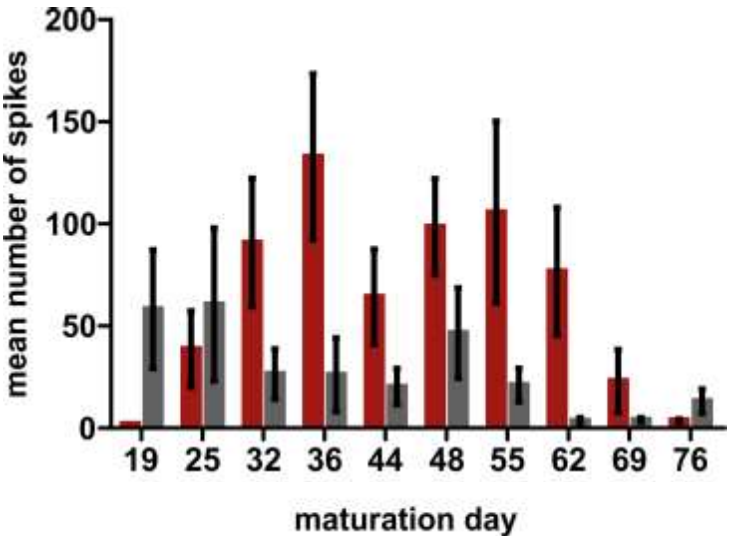


Spontaneous activity of patient (β3 L10P)-derived sensory neurons is enhanced in patch-clamp recordings. (Left) Example of spontaneous APs of a β3 L10P-sensory neuron and (right) percentage of spontaneously active patient- and control-derived nociceptors.

Dr. Eberhardt

Furthermore we could increase the purity of the neuronal culture during maturation combining surface marker based sorting strategies with mitotic inhibition as important prerequisite for further RNA-based approaches to identify novel pain associated target genes.

Grown on MEA plates sensory neurons of the patient carrying the β3 mutation displayed an increased number of active electrodes and increased electrical activity per active electrode (mean number of spikes) over a broad range of the maturation period as a sign of enhanced spontaneous activity. In whole cell patch-clamp recordings the overall morphology of the action potential (AP) compared to controls was not significantly altered. However, 21% of patient-derived sensory neurons were spontaneously active compared to none in the control population and showed a significant decrease in current threshold to trigger action potentials and an increase in evoked electrical activity.



Patient (β3 L10P)-derived nociceptors show increased spontaneous activity in MEA recordings (plotted as mean number of spikes per active electrode during a recording of two minutes).

In summary patient (β3 L10P)-derived sensory neurons show signs of hyperexcitability in patch-clamp and MEA recordings and mirror increased spontaneous firing found in the C-fibers of both patients in microneurography recordings. It is therefore likely that β subunits have a regulatory function in the pathophysiology of pain which has to be further elucidated.

Contact:
Dr. Eberhardt
phone: + 49 9131 85 42465
e-mail: esther.eberhardt@uk-erlangen.de

Publications during funding period

Namer B, Schmidt D, Eberhardt E, Maroni M, Dorfmeister E, Kleggetveit IP, Kaluza L, Meents J, Gerlach A, Lin Z, Winterpacht A, Dragicevic E, Kohl Z, Schüttler J, Kurth I, Warncke T, Jorum E, Winner B, Lampert A (2019) Pain relief in a neuropathy patient by lacosamide: Proof of principle of clinical translation from patient-specific iPS cell-derived nociceptors. EBioMedicine 39:401-408

Maroni M, Körner J, Schüttler J, Winner B, Lampert A, Eberhardt (2019) β1 and β3 subunits amplify mechanosensitivity of the cardiac voltage-gated sodium channel Nav1.5. Pflugers Arch. 471(11-12):1481-1492

J67 - Progress Report

01/01/2018 - 30/06/2020

Oncology

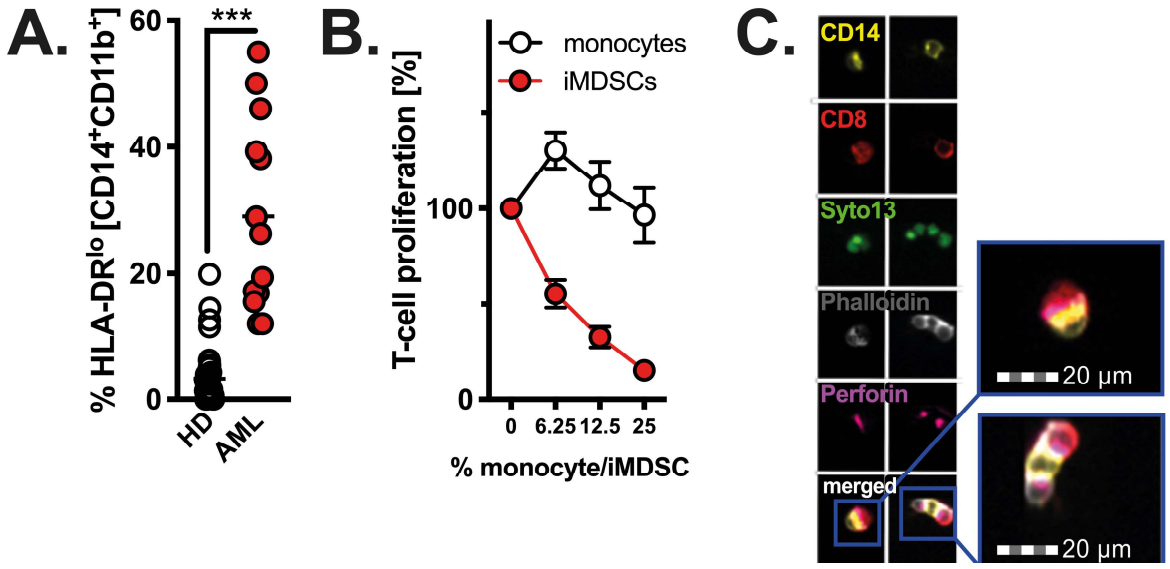
Metabolic reprogramming of AML MDSCs

PD Dr. Dr. Regina Jitschin, Department of Medicine 5 - Hematology and Medical Oncology

Acute myeloid leukemia (AML) is the most common acute leukemia amongst adults. Emerging evidence suggests that immune alterations favor leukemogenesis and relapse. Myeloid derived suppressor cells (MDSCs) have gained momentum as mediators of immune escape. We aim to decipher interconnections between metabolic reprogramming and MDSC abundance and to unravel the role of AML-derived exosomes in this context. Understanding those mechanisms is key for improving immune-based therapeutic approaches.

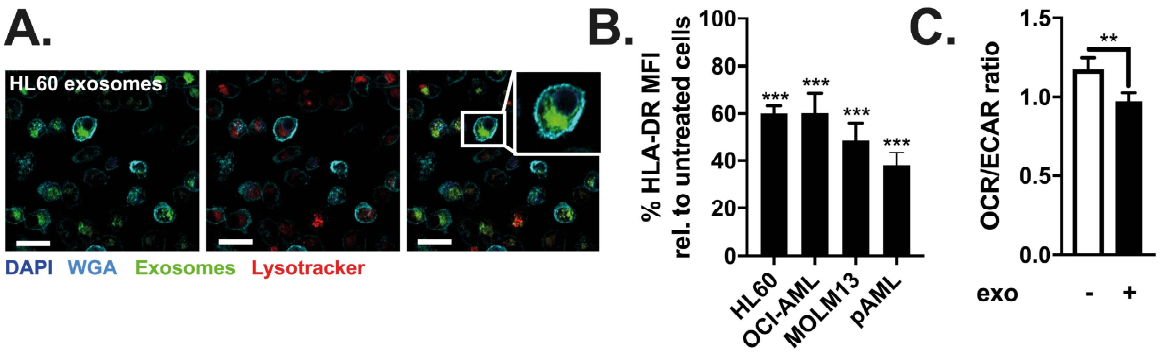
MDSCs are a heterogeneous cell population morphologically resembling either monocytes or granulocytes and sharing some key features including myeloid origin, aberrant (immature) phenotype, and immunosuppressive activity. Increasing evidence suggests that accumulating MDSCs are involved in hampering anti-tumor immune responses and immune-based therapies.

First, we assessed the presence of circulating CD14+ cells that co-express CD33 but lack HLA-DR expression (HLA-DRlo) in patients with newly diagnosed AML. These monocytic cells represent one of the best-defined human MDSC subsets. Frequency of CD14+HLA-DRlo cells was significantly increased in untreated AML patients. Purified CD14+HLA-DRlo cells suppressed in vitro T-cell proliferation in a concentration-dependent manner allowing us their denomination as MDSCs. Primary AML blasts and human AML cell lines (THP, OCI-AML, and HL-60) (and their respective exosomes) induced HLA-DRlo cells from healthy donor-derived monocytes that were T-cell suppressive and expressed the immune regulatory indoleamine-2,3-dioxygenase (IDO). Des-



(A) CD14+HLA-DRlo cells in AML patients and healthy controls. (B) T cell-suppressive activity of monocytes and AML-induced MDSCs was evaluated in co-cultures with autologous T-cells using FACS. (C) FACS-imaging of CD8+ T-cells (red) conjugated with CD14+CD33+ MDSCs (yellow). ***, p<0.001.

PD Dr. Dr. Jitschin



(A) HL60-derived exosomes (green) are taken up by healthy control-derived monocytes as assessed by confocal microscopy. (B) HLA-DR levels as assessed on monocytes treated with AML cell line- and primary (p)AML-derived exosomes. (C) AML-exosomes skew the ration of OCR (=respiration) and ECAR (=glycolysis).

pite their T-cell suppressive activity, MDSCs could be targeted by T-cell engaging anti-CD33/CD3 bispecific BiTE® antibodies (AMG 330) with well-documented anti-leukemic activity. Taken together, our results suggest that anti-CD33/CD3 bispecific BiTE® antibody constructs may achieve anti-leukemic efficacy not only through direct T cell mediated cytotoxicity against AML blasts but also through circumventing immune evasion via MDSCs targeting. Although therapeutic targeting of MDSCs in patients has not yet been successfully accomplished, bystander killing of CD33+ MDSCs via anti-CD33/CD3-bispecific BiTE® antibody constructs could represent a very promising approach to increase the anti-leukemic T-cell response in AML patients and to reverse immune evasion.

Next, we revealed that conventional monocytes readily take up AML-derived exosomes and subsequently undergo MDSC differentiation. They acquire an CD14+HLA-DRlo phenotype and express the immunomodulatory IDO. Molecular markers such as S100A8/9 and cEBPb that are proposed as MDSC-defining features were elevated in response to AML-exosomes. In addition, we observed a phenotypic/functional overlap with characteristics of (rather leukemia-supporting) M2-like monocytes/macrophages, which is in line with previ-ous reports on AML-promoted M2-polarization. We identified the critical role of the Akt/mTOR pathway for the AML-exosome-induced phenotypical and functional transition of monocytes. Upon AML-exosome treatment we documented a metabolic skewing away from OXPHOS and fatty acid utilization towards aerobic glycolysis. In fact, AML-exosome-triggered glycolysis rendered MDSCs more susceptible towards interference within their glucose metabolism, which is remarkable since AML-exosomes alone promoted survival and therefore could represent a strategy to metabolically target MDSCs.

Contact:
PD Dr. Dr. Jitschin
phone: + 49 9131 85 43113
e-mail: regina.jitschin@uk-erlangen.de

Publications during funding period

Jitschin R, Saul D, Braun M, Tohumeken S, Völkl S, Kischel R, Lutteropp M, Dos Santos C, Mackensen A, Mougiakakos D (2018) CD33/CD3-bispecific T-cell engaging (BiTE®) antibody construct targets monocytic AML myeloid-derived suppressor cells. Journal of Immunotherapy of Cancer 6(1):116. doi: 10.1186/s40425-018-0432-9

J68 - Progress Report

01/10/2017 - 31/03/2020

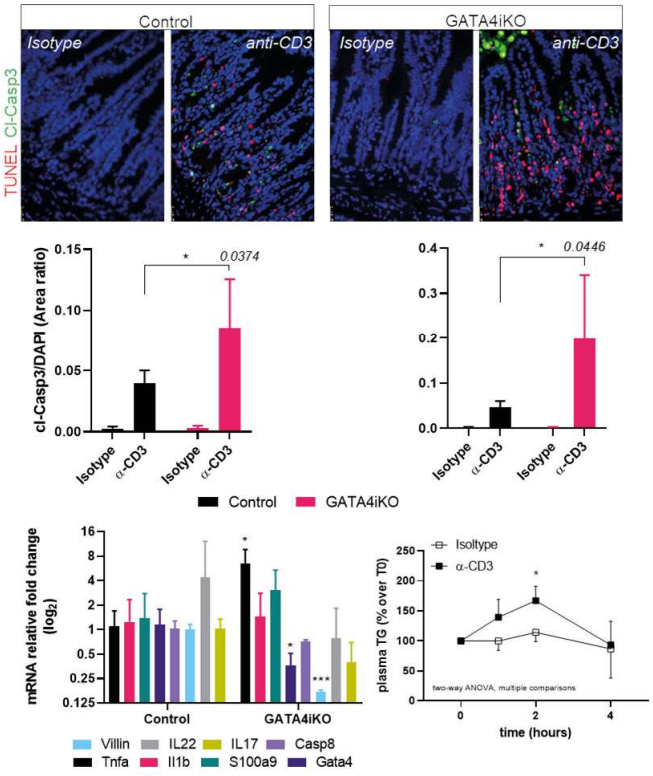
Oncology

Role of GATA4 in Intestinal Inflammation & Cancer

Dr. Jay V. Patankar, Department of Medicine 1 – Gastroenterology, Pneumology and Endocrinology

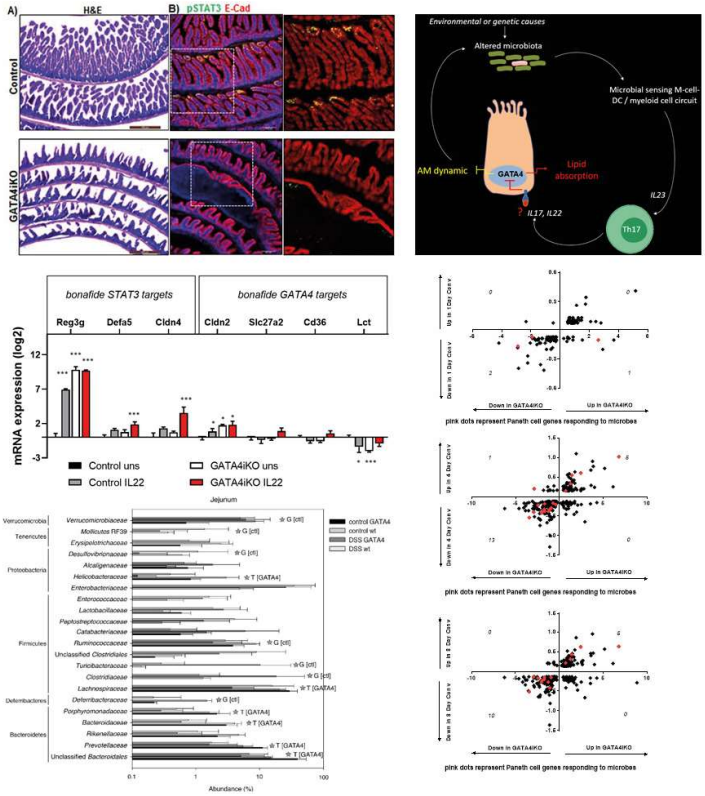
A key endodermal transcription factor involved in intestinal differentiation is GATA4 with an unrecognized role in intestinal pathologies. Our previous work and recently published literature indicates that GATA4 regulates epithelial inflammatory and metabolic transcription and is altered in cancer. Intron retention (IR) is a novel post-transcriptional modification of mRNA that frequently affects tumor suppressor genes. We have now identified GATA4 as a key regulator of intestinal microbiota composition, regulation of enterocyte metabolic function and a novel target of IR.

We previously showed that GATA4 is crucial for intestinal metabolic function. We now show that intestinal T-cell activation directly affects GATA4-mediated metabolic function. On the other hand, mice lacking intestinal epithelial GATA4 (GATA4iKO) have reduced phosphorylation of epithelial STAT3 that was associated with lower mRNA expression of epithelial STAT3 target Adora2b, a well-known risk gene for inflammatory bowel disease. The activation of T-cells or induction of small intestinal damage by non-steroidal anti-inflammatory drugs was also more severe in the GATA4iKO group compared with controls evidenced by increased inflammatory gene expression. It is known that epithelial STAT3 phosphorylation is regulated by the sensing of gut microbiota by specific T cells in the gut. To test whether the absence of STAT3 phosphorylaiton could stem from altered gut microbiota, we next measured the levels of various microbial species in the intestines of control and GATA4iKO mice. Our analysis has revealed that the jejunum GATA4iKO mice had a significantly different composition than control mice (p=0.02, permANOVA). The jejunum of GATA4iKO mice is depleted in several families, including several known mucus-associated groups (Verrucomicrobiaceae, Deferribacteraceae, and Desulfovibrionaceae), indicating that the normal mucus-associated microbiota is disrupted in these mice. These data indicate that epithelial GATA4 is a critical regulator of the normal microbial dynamic in the gut. For our second objective, we aimed at outlining the role of GATA4 in intestinal wound healing and resolution in vivo. For this, we took advantage of the DSS induced intestinal injury model. We found that GATA4iKO mice have a significant reduction in survival on 3.5% DSS. When DSS is removed to allow for



Worsening of small intestinal pathology in GATA4iKO mice upon T cell activation correlates with higher apoptosis rates, higher inflammatory cytokine expression and increased triglyceride leakage.

Dr. Patankar



Aberrant antimicrobial response and altered gut microbiota in the small intestine of GATA4iKO mice. Gene expression of small intestine lacking GATA4 positively correlates with that of germfree mice exposed to conventional microbiota.

recovery, GATA4iKO mice show a delay in the normal course of recovery with higher fecal blood loss ($p<0.05$, t-test) and a significantly higher endoscopic damage score ($p = 0.013$, t-test). This was accompanied with a higher infiltration of F4/80 positive macrophages in the damaged area. These results support our hypothesis that epithelial GATA4 is crucial for intestinal recovery and repair. Our third objective links the homeostatic functions of GATA4 to its putative role in intestinal carcinogenesis. Here, we have identified that intron 4 of the GATA4 gene is affected by a specific intron retention (IR) event in two colorectal cancer mouse models and have followed it up in patient samples where we have identified IR in of a corresponding non-conserved intron, intron 6, of the human GATA4 gene in colorectal cancer samples. Both of these introns introduce premature stop codons, however, the transcript stability is not affected. Future experiments will investigate the coding potential of the GATA4 transcript in these cancer tissues.

Contact:
Dr. Patankar
phone: + 49 9131 85 35791
e-mail: jay.patankar@uk-erlangen.de

Patents/ Licenses during funding period

Modulation of cyp8b1 for the prevention and treatment of liver fibrosis and metabolic disorders. Inventors: Michael R. Hayden Jay PATANKAR, WO2017000058A8, 2017-10-19

Publications during funding period

Größwein S, Lindemann A, Mahajan A, Maueröder C, Martini E, Patankar J, Schett G, Becker C, Wirtz S, Naumann-Bartsch N, Bianchi ME, Greer PA, Lochnit G, Herrmann M, Neurath MF, Leppkes M (2019) Citrullination Licenses Calpain to Decondense Nuclei in Neutrophil Extracellular Trap Formation. Front Immunol.10:2481

J69 - Progress Report

01/09/2018 - 28/02/2021

Effect of HIV on pre-existing vaccine immunity

Dr. Christiane Krystelle Nganou Makamdop, Institute of Clinical and Molecular Virology

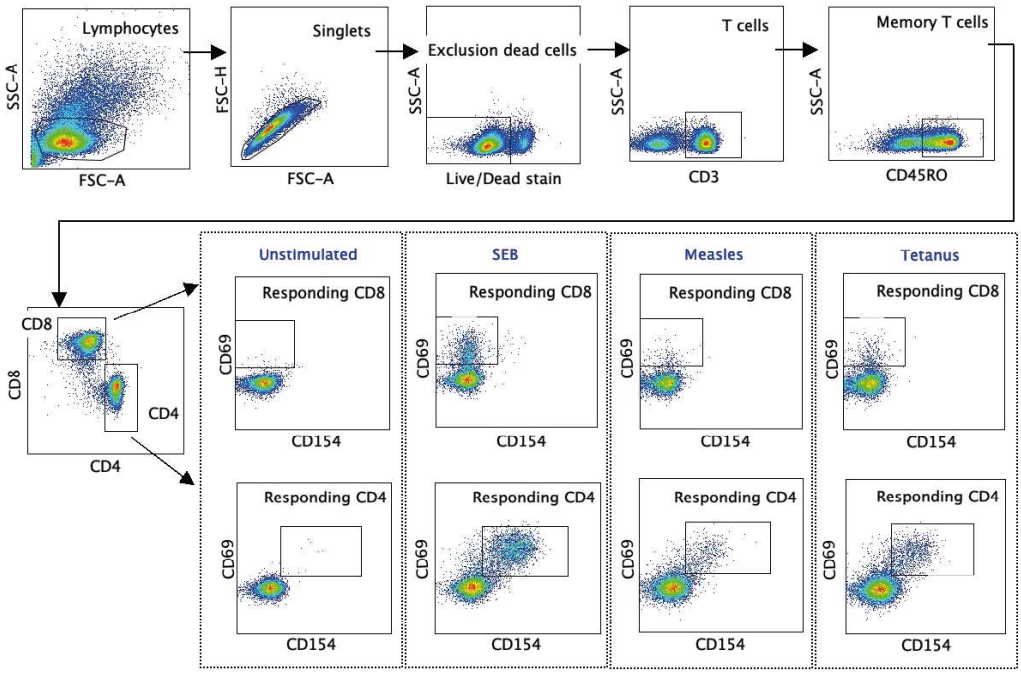
With 36.9 million infected persons worldwide and no vaccine in sight, HIV infection remains a threat to global health. Depletion of CD4 T cells, microbial translocation and chronic inflammation are, to name a few, mechanisms by which the virus impairs overall immunity. This has serious implications for the maintenance of antigen-specific responses, even in persons on antiretroviral therapy (ART). This project aims at studying the quality of vaccine-induced immunity HIV-infected persons on ART.

Approach

So far we have recruited 35 HIV-uninfected and 34 ART-treated HIV-infected participants. Markers of inflammation and microbial translocation are being assessed in plasma in order to gauge the extent of immune imbalance in the HIV-infected group. In addition, peripheral recall T cell responses to measles virus (MV) and tetanus toxoid (TT) in previously vaccinated HIV-infected and uninfected persons are being assessed by means of in vitro stimulation and Flow cytometry.

Preliminary work

Our preliminary work shows that despite ART, HIV-infected study participants have more pronounced T cell activation (as defined by HLA-DR expression), elevated levels of inflammation (as defined by among others IL-6 and IL-8 plasma concentrations) and ongoing microbial translocation (as defined by sCD14 and IFABP plasma concentrations). These data confirm that ART has not fully restored immune dysfunctions in our study participants.

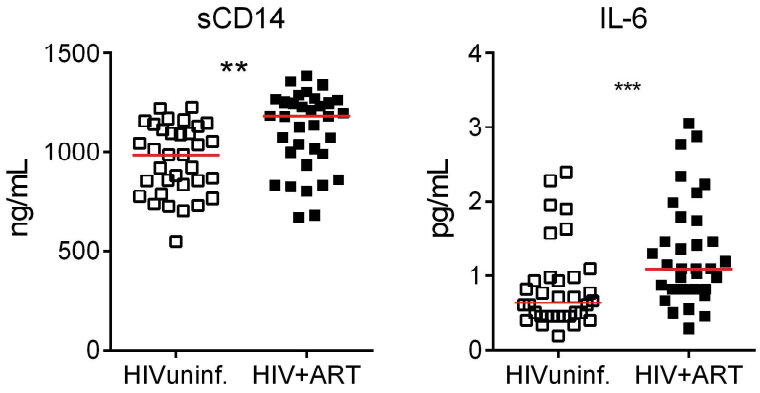


Gating strategy for cell sorting. Memory CD4 and CD8 T cells responding to measles and tetanus toxoid antigens are sorted based on CD154 and CD69 expression. SEB and unstimulated conditions are positive and negative controls respectively.

Dr. Nganou Makamdop

Next, recall T cells responses are being assessed by stimulation of PBMCs with TT and MV antigens followed by measurements of cytokine production, T cell activation and proliferation. These experiments show a generally lower percentage of antigen responding T cells in the HIV-infected group compared to HIV-uninfected group. In order to elucidate mechanisms of impaired T cell responses in HIV-infected participants, we are currently sorting antigen responding T cells that will further undergo transcriptome analysis (Sorting conducted at the core unit 'cell sorting with immune monitoring' of the Faculty of Medicine). Although the levels of antigen-responding T cells are generally lower in the HIV-infected group, we have established a protocol that generates sufficient cell sort yield for downstream applications.

Thus far, measurement of MV- or TT-binding IgGs in plasma shows no major difference between groups. Presumably, additional methods such as neutralization capacity or the use of complementary parameters (IgG subclass ratios, time from last boost for infight into the decay rate or CD4 recovery) may be included for a formal conclusion on these antibody responses in our study participants



Persistent inflammation in the HIV-infected study participants on ART. Plasma concentrations of sCD14 and IL-6 were measured by ELISA. Red lines represent median values.

Next steps

Given that persistent inflammation is marked by elevated levels of innate cytokines, we will next evaluate responses of innate cells in our study participants. In addition, we will further analyze antibody responses by means of neutralization assays as well as specificity at the subclass level to infer a functional relationship between assessed T cell responses and serological responses.

Contact:
Krystelle Nganou
Phone: + 49 9131 85 26783
email: krystelle.nganou@uk-erlangen.de

Publications during funding period

none

Gene discovery in kidney disease

Dr. Tilman Jobst-Schwan, Department of Medicine 4 - Nephrology and Hypertension

The genetic background of chronic kidney disease (CKD) in adults is insufficiently investigated. We perform genetic testing on local adult patients with CKD to identify novel monogenic causes of CKD. To prove deleteriousness of mutations identified, functional studies including RNA-Seq are conducted in primary skin fibroblasts or human urinary primary tubular cells (hUPT) of the patients. Candidate genes are further investigated, inter alia, in a zebrafish loss-of-function animal model.

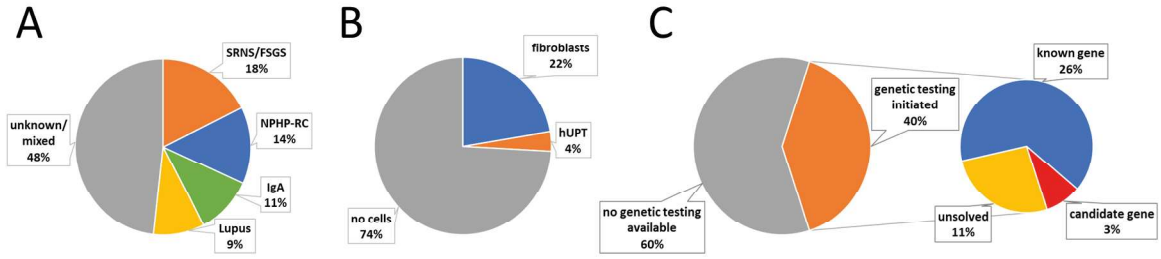
The local cohort of CKD patients with suspected genetic background

Since the beginning of the funding period in October 2018, 34 additional CKD patients with potentially genetic background have been recruited to the study with the entire cohort containing 85 families now. 15 patients presented with steroid resistant nephrotic syndrome/focal segmental glomerular sclerosis (SRNS/FSGS; 18%), 12 with a nephronophthisis related ciliopathy (NPHP-RC; 14%), 9 with IgA nephritis (11%) and 8 with renal involvement of Lupus erythematodes (9%). The remaining patients (48%) showed different other forms of CKD, but most were diagnosed with CKD of unknown origin. From 19 patients, primary skin fibroblasts have been obtained (22%). Due to a high degree of already transplanted patients in the recruited cohort with excretion of donor tubular cells instead of patient derived cells, hUPT have been generated from only 3 patients (4%).

Genetic testing has been initiated for 34 families (40%;). Thereby, 25 families have been solved for a known disease gene (26%). For 3 families, novel candidate genes have been established (3%). 9 Families (11%) remain unsolved to date. The high solving rate of 65% of all tested patients confirms the reliability of our pretest assessment for a potential genetic disease background.

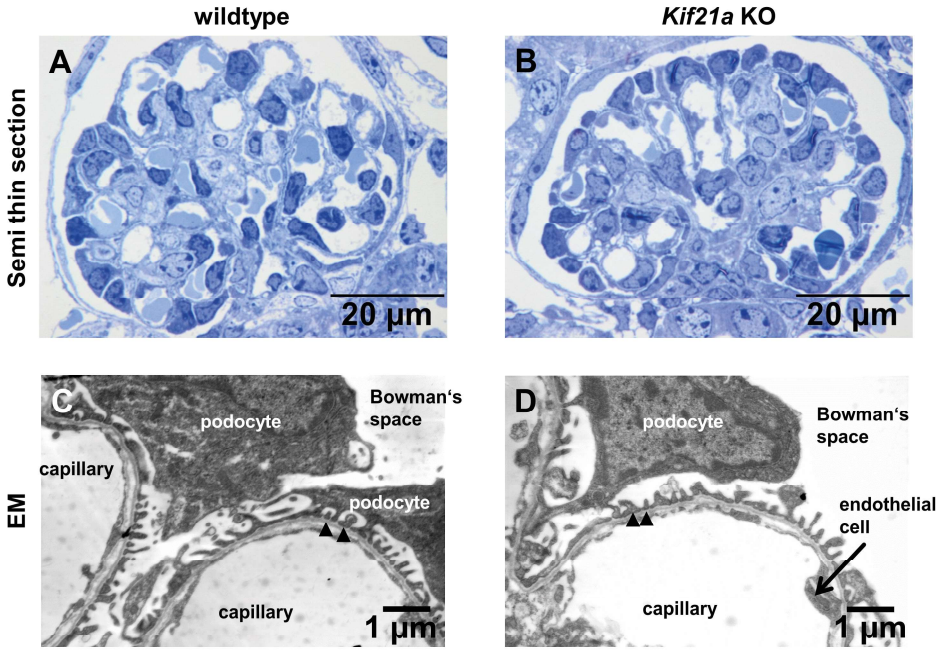
Analysis of the candidate gene *KIF21A*

In a patient with SRNS from a consanguineous family with multiple affected members, we identified a homozygous obligatory splice site mutation in the gene *KIF21A* (NM_017641.3, c.3633-1G>A,p.0?) by Whole Exome Sequencing. qPCR experiments showed inconsistent results. Primary skin fibroblasts were obtained from the patient and her healthy heterozygous parents. Migration experiments and immunofluorescence-based analysis of the actin cytoskeleton did not show alterations compared to the



Description of the CKD cohort. (A) Composition of the patient cohort; abbreviations are defined in the main text. (B) Primary cells generated from CKD patients. (C) Solve rates for genetic testing with discovery of candidate genes in 3 families.

Dr. Jobst-Schwan



Histological analysis of *Kif21a* KO mouse kidneys. Toluidine blue staining reveals normal morphology in wildtype (wt; A) and KO glomeruli (B). Electron microscopy shows normal podocyte slit diaphragm morphology in wt (C) and KO glomeruli (D) at E18.5.

heterozygous parents and healthy donors. Transcriptional profiling of the patient derived primary cells has been initiated. As the fibroblast phenotype did not show any obvious phenotype, we generated induced pluripotent stem cells from these fibroblasts in an intradepartemental collaboration with the laboratory of Prof. Mario Schiffer. These will be differentiated into podocytes, and analyzed for a cell type dependent phenotype.

In collaboration with the laboratory of Elisabeth Engle, Boston Children's Hospital, USA, and Prof. Christoph Daniel from the Department of Nephropathology in Erlangen, we analyzed kidneys of *Kif21a* knock-out (KO) mice at E18.5 (homozygous *Kif21a* KO mice die at P1/P2). We did not find any structural alterations of glomeruli and the slit diaphragm by light- and electron microscopy. However, the early most likely non-renal cause of lethality in these animals does not allow for a sufficient extra uterine observation period. Further cell culture and zebrafish studies may elucidate if *KIF21A* can be maintained as a candidate gene for SRNS.

Contact:
Dr. Jobst-Schwan
phone: + 49 9131 85 42956
e-mail: tilman.jobst-schwan@uk-erlangen.de

Publications during funding period

none

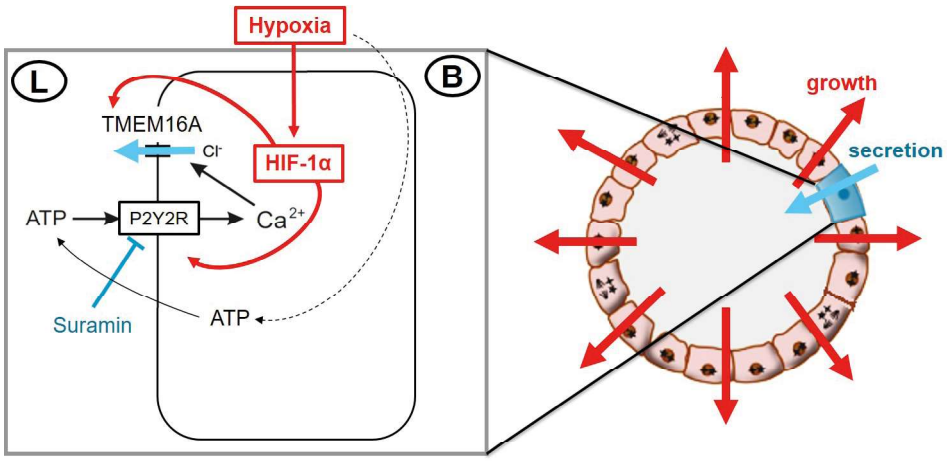
P2Y2R-dependent cyst growth in ADPKD

Dr. Andre Kraus, Department of Internal Medicine 4 - Nephrology and Hypertension

The main aim of our project is to descramble the precise role of the ATP-activated purinergic receptor P2Y2R in the context of Ca^{2+}-dependent Cl^--secretion as a main course of cyst growth in Autosomal Dominant Polycystic Kidney Disease. We are analysing the effect of Suramin (P2R inhibitor) as a potential drug and the impact of genetic deletion of P2Y2R in a PKD1-KO mouse model. In addition we test for ATP-dependent effects by performing micropuncture experiments in an in vitro cyst model.

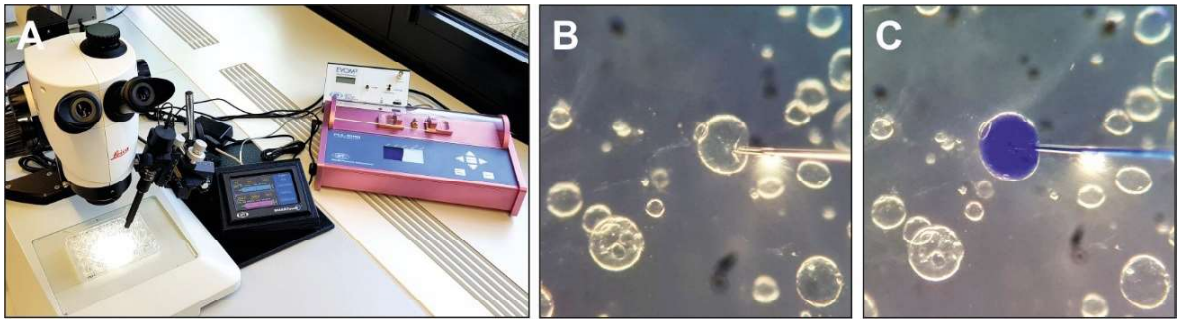
Background

Autosomal dominant polycystic kidney disease (ADPKD) is the most common monogenic renal disease affecting ~1 in 1000 persons and resulting in chronic kidney failure. As a consequence, kidney replacement therapy is needed in more than half of the affected individuals by the age of ~55 years. In 78% of the cases, a mutation in the PKD1 gene is responsible for the disease, which is characterized by the development of a large number of bilateral renal cysts mainly originating from the collecting ducts and which grow continuously over decades. The driving force for cyst growth is the fluid secretion into the cyst lumen. We could show that calcium-activated chloride secretion, which acts synergistically with cAMP mediated secretion is significantly involved. Calcium-activated chloride secretion is typically induced by extracellular ATP, which stimulates purinergic receptors like Gq-coupled P2Y receptors or ATP-gated P2X receptors. ATP has been shown to accumulate within the cyst fluid reaching concentrations of up to 10µM, which is highly sufficient to activate the mentioned purinergic receptors. The activation results in an increase of intracellular calcium which then activates calcium-activated chloride channels. In line with these data, we could recently show, that the purinergic receptor P2Y2R is expressed in the cyst-lining cells in mouse and human ADPKD.



The role of P2Y2R and calcium-activated chloride secretion in cyst growth.

Dr. Kraus



Setup for cyst microinjection

Generation of PKD1 – P2Y2R double knockout mice to test for the role of P2Y2R for in vivo cyst progression

We have been able to import and undergo embryo transfer of P2Y2R floxed mice to our lab. Those mice have been crossed with our established inducible tubule-specific PKD1 knockout mouse model and we will now analyse the impact of additional knockout of P2Y2R on cyst progression. To further analyse the potency of therapeutic inhibition of P2Y2R for cyst progression, we will administer Suramin 5x/week to our induced PKD1 knockout mice. Suramin is commonly used as an inhibitor for purinergic receptors.

Impact of luminal ATP on *in vitro* cyst growth

Since ATP accumulation within the cyst fluid has been proposed to be the driving force for calcium-activated chloride secretion and cyst enlargement, we further extended our experimental *in vitro* cyst model in order to test for apical ATP-dependent effects on cyst growth as well as mechanisms promoting apical ATP release. Therefore, we have installed a micro puncture aperture. Now, we are able to either inject substances like ATP directly into the cyst lumen *in vitro.* In addition, cyst growth is monitored by the use of a live cell imaging unit. Currently we are injecting ATP luminaly into the cyst and measuring cyst growth under different conditions.

Contact:
Dr. Kraus
phone: + 49 9131 85 40110
e-mail: andre.kraus@uk-erlangen.de

Invited lectures

SFB1350 annual retreat in November 2019 in Beilngries on “Slowing polycystic kidney disease by inhibition of HIF-1α-dependent- and calcium-mediated secretory signaling pathways”

Awards

Rainer-Greger-Promotionspreis 07.11.2019 from the Deutsche Gesellschaft für Nephrologie e. V. (DGfN) for the best dissertation in the field of kidney- und hypertension research.

Publications during funding period

Kraus A, Peters DJ, Klanke B, Weidemann A, Willam C, Schley G, Kunzelmann K, Eckardt KU, Buchholz B (2018) HIF-1α promotes cyst progression in a mouse model for autosomal dominant polycystic kidney disease. Kidney Int. ;94(5):887-899. doi: 10.1016/j.kint.2018.06.008. Epub 2018 Aug 30. PMID: 30173898

J73 - Progress Report

16/09/2018 - 15/03/2021

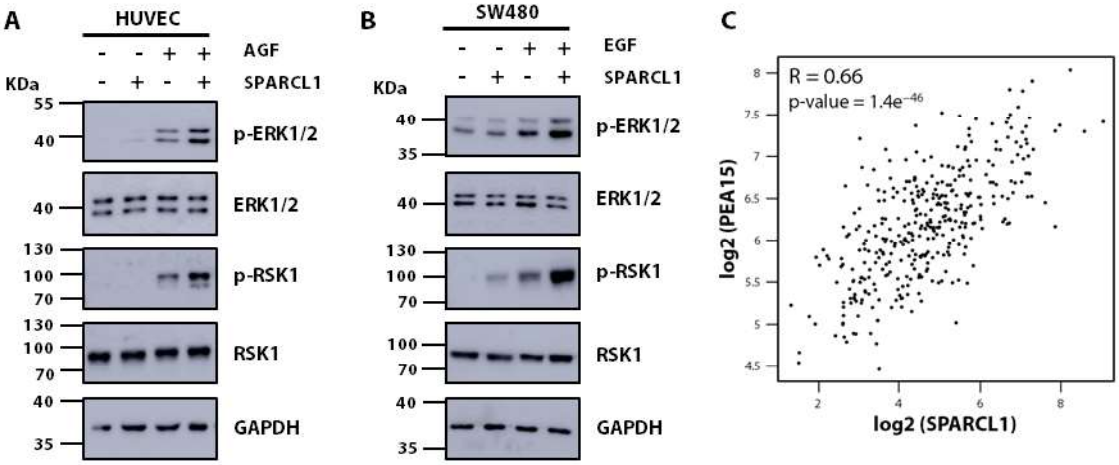
Oncology

Intracellular signaling by SPARCL1 in colon cancer

Dr. Clara Tenkerian, Department of Surgery

The tumor microenvironment (TME) plays a pivotal role in tumorigenesis, prognosis and therapy. SPARCL1 is a vascular derived anti-tumorigenic factor that counteracts CRC tumorigenesis in a TME-dependent manner. Preliminary results indicate that SPARCL1 regulates ERK phosphorylation and subcellular localization in endothelial and CRC cells. This project aims to elucidate the signaling pathways by which SPARCL1 transmits its anti-proliferative and anti-angiogenic functions, mainly focusing on ERK.

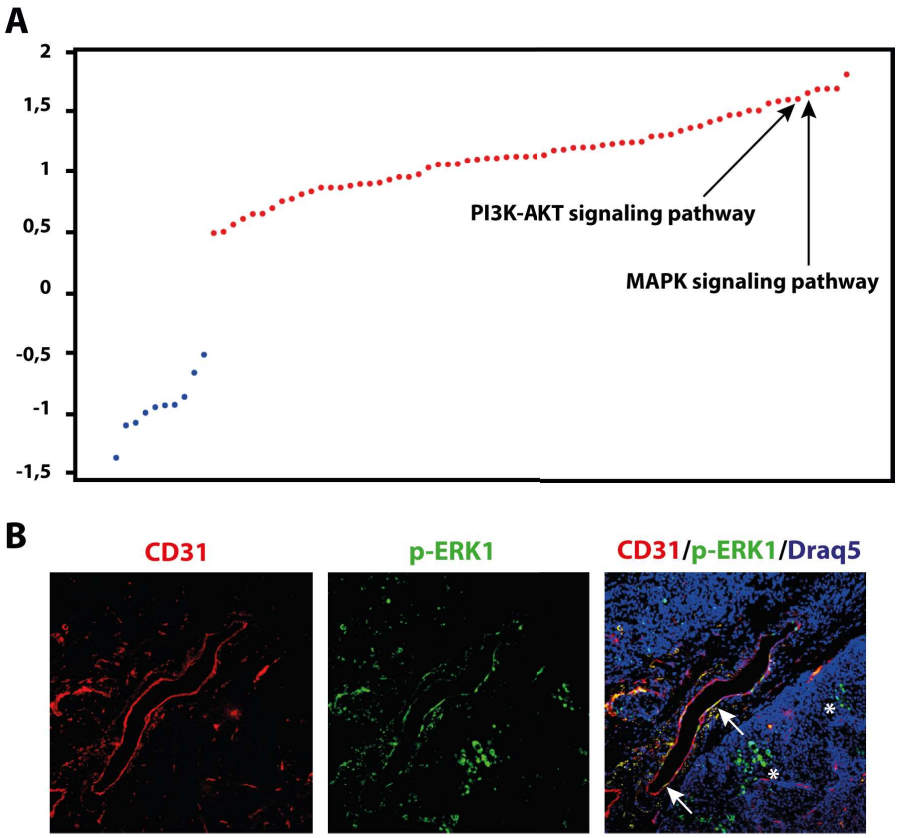
Preclinical success in cancer drug development often does not translate into clinical response in patients, suggesting that components of the TME contribute substantially to treatment outcome. SPARCL1 is a vascular-derived regulator of TME-dependent plasticity of tumor endothelial cells (TECs) in colorectal carcinoma (CRC). Specifically, SPARCL1 expression is associated with a Th1-TME, which confers a survival benefit in CRC, and is lost in non-Th1-TME. The heterogeneity of TECs may severely impair therapy approaches in cancer, potentially through its impact on signaling pathways. RAS-ERK pathway activation occurs commonly in CRC and as such is under intense scrutiny as an obvious therapeutic target. We have identified a SPARCL1-dependent regulation of ERK signaling in both endothelial cells and CRC cell lines. Preliminary results indicated that SPARCL1 phosphorylates ERK preferentially in the cytoplasm. To further characterize the mechanism of SPARCL1-mediated ERK regulation, we investigated the effects of SPARCL1 on downstream targets of ERK. Over 250 distinct substrates of ERK1/2 have been identified. The p90 ribosomal S6 kinase 1 (RSK1) and the cytosolic Phospho-Lipase A2 (cPLA2) are substrates of ERK phosphorylated specifically in the cytoplasm. We observed increased phosphorylation of these cytoplasmic ERK targets in the presence of SPARCL1 when stimulated with growth factors that activate ERK: angiogenic growth factors (AGF) in endothelial cells and epidermal growth factor (EGF) in CRC cell lines. We hypothesize that ERK is retained in the cytoplasm in the presence of SPARCL1 due to inhibition of its re-



SPARCL1 phosphorylates ERK in both endothelial cells and CRC cell lines and activates cytoplasmic targets of p-ERK such as RSK1 (A, B). This is potentially mediated through its interaction with PEA15, a scaffold protein that anchors ERK in the cytoplasm (C).

Dr. Tenkerian

(A) The MAPK signaling pathway is enriched in TECs isolated from CRC patients with a Th1-TME compared to the control group. (B) ERK phosphorylation is observed in both endothelial cells (arrows) and tumor cells (asterisk) in colon cancer tissues.



lease from cytoplasmic anchors upon phosphorylation. The Proliferation and Apoptosis Adaptor Protein 15 (PEA15) is a scaffold protein that tethers ERK in the cytoplasm in quiescent cells. Gene expression analysis of CRC patient data from the TCGA database (n= 367) shows a positive correlation in the expression of SPARCL1 and PEA15 with an R coefficient of 0.66, encouraging further investigation of this protein.

To determine whether the SPARCL1-mediated regulation of ERK observed in vitro occurs in patients, we performed enrichment analysis of differentially regulated genes in TECs isolated from CRC patient tumors and divided between Th1-TME and control-TME groups. The MAPK signaling pathway was shown to be enriched in TECs with Th1-TME compared to the control group. Furthermore, given that the effects of SPARCL1 observed in vitro extended beyond endothelial cells to regulate ERK phosphorylation in tumor epithelial cells as well, patient colon tissues were stained to observe p-ERK localization. We observed both endothelial (co-localization with CD31) and epithelial-associated ERK phosphorylation. In a next step, the contribution of SPARCL1 to the regulation of epithelial and endothelial ERK and its targets will be evaluated in an established cohort of CRC patients with high and low expression of SPARCL1.

Contact:
Dr. Tenkerian
phone: + 49 9131 85 39524
e-mail: clara.tenkerian@uk-erlangen.de

Invited lectures

DFG Research Unit 2438 Symposium „Cell Plasticity in Colorectal Carcinogenesis“, 19.-20.09.2019, Frankfurt, Tumor microenvironment-dependent vascular plasticity in colorectal cancer

Publications during funding period

none

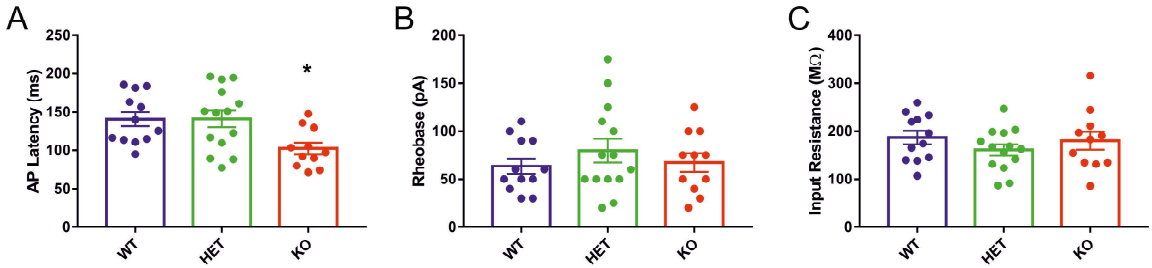
The role of CtBP1 in hippocampal and cortical neuroplasticity

Dr. Seda Salar, Department of Psychiatry and Psychotherapy

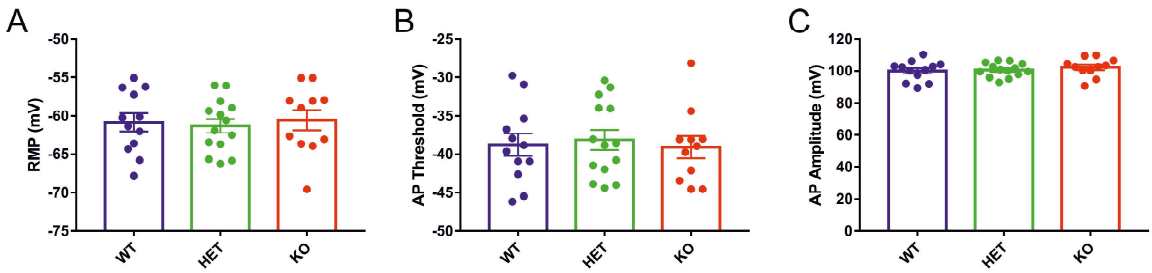
Neuronal excitability alterations lead to both physiological (i.e., memory) and pathological plasticity (i.e., seizures). Long-lasting neuroplastic changes require gene expression and therefore, intracellular mediators linking neuronal activity to gene expression. C-terminal binding protein 1 (CtBP1) is a transcriptional corepressor, shuttling between nucleus and presynapse depending on the neuronal activity. We aim to elucidate its role in hippocampal and cortical neuroplasticity.

A number of intracellular cascades or molecules connect neuronal activity to gene expression. The balanced transmission of this information enables the long-term storage of memories which would otherwise lead to maladaptive plasticity. CtBP1 regulates gene expression and its location is dependent on the neuronal activity. In the basal state, CtBP1 is in the nucleus, a part of a gene silencing complex, repressing the expression of genes that are involved in both physiological and pathological neuroplasticity such as BDNF and CamKII. Following neuronal activity, CtBP1 translocates to presynapses and is anchored to scaffolding proteins.

We hypothesize that CtBP1 may represent a link between physiological and pathological plasticity. We aim to characterize its function in neuroplasticity by using acute brain slices from CtBP1 knock-out (KO), heterozygote (HET) and wild-type (WT) mice. Due to the expression pattern of CtBP1, we first focused on hippocampal CA1 region and pyramidal cells.

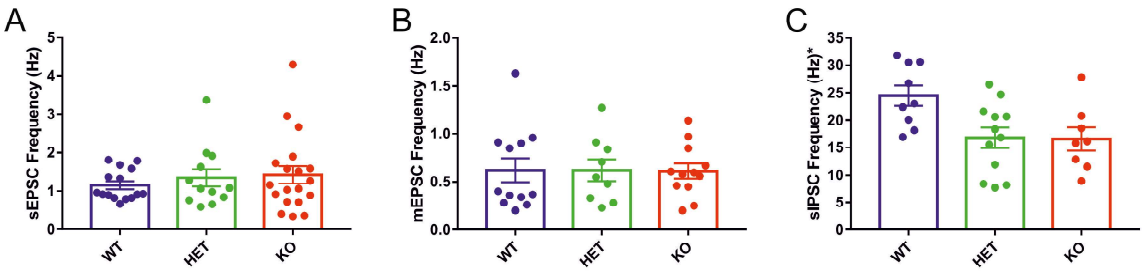


Comparison of intrinsic cell properties between WT, HET and KO-1. A) action potential latency, B) rheobase, C) input resistance.



Comparison of intrinsic cell properties between WT, HET and KO-2. A) resting membrane potential, B) action potential threshold, C) action potential amplitude.

Dr. Salar



Comparison of AP-dependent and -independent basal transmission between WT, HET and KO-2. A) sEPSC frequency, B) mEPSC frequency, C) sIPSC frequency. *preliminary data.

Intrinsic neuronal properties

We investigated the intrinsic neuronal properties such as resting membrane potential (RMP), input resistance, action potential (AP) threshold and number, and the latency and amplitude of the first APs. The latency to the first AP induction was found to be significantly lower in KOs compared to HETs and WTs when cell soma was activated by injection of increasing current steps ($p<0.03$, Kruskal-Wallis followed by Dunn's multiple comparison test). Other parameters showed no differences between groups.

Lower latency could be an indication of increased conductance in KOs, although no difference was seen in input resistance. On the other hand, it might also indicate increased pyramidal cell excitability in KOs given that the rheobase was similar between the groups. We, therefore, next examined basal synaptic neurotransmission.

Basal synaptic transmission

AP-mediated and -independent spontaneous excitatory postsynaptic currents (sEPSC and mEPSC, respectively) were investigated. Frequencies, frequency distributions and amplitudes of spontaneous currents were measured from cells that were voltage clamped at -70 mV. None of the measured parameters showed a difference between groups indicating an unaltered basal excitatory synaptic transmission. Increased excitability could also be due to a reduction in the inhibitory tone. To this end, we have started to measure spontaneous postsynaptic inhibitory currents (sIPSC and mIPSCs). Our preliminary results showed tendency towards lower sIPSC frequencies and amplitudes. Further experiments will reveal whether basal inhibitory synaptic transmission and overall inhibitory tone is affected in CtBP1 KO mice.

Overall, our results point out the compensatory changes during basal state. We will investigate alterations in activated physiological and pathological neuronal states. Our results may shed light on regulation of neuronal activity or probable compensatory mechanisms against these alterations.

Contact:
Dr. Salar
phone: + 49 9131 85 44883
e-mail: seda.salar@uk-erlangen.de

Publications during funding period

Salar S, Guhathakurta D, Marx Hoffmann L (2019) Differential contribution of pyramidal cells and interneurons to activity-dependent gene transcription changes. J Neurophysiol. 122(6):2203-2205. Doi: 10.1152/jn.00089.2019

J75 - Progress Report

16/10/2018 - 15/04/2021

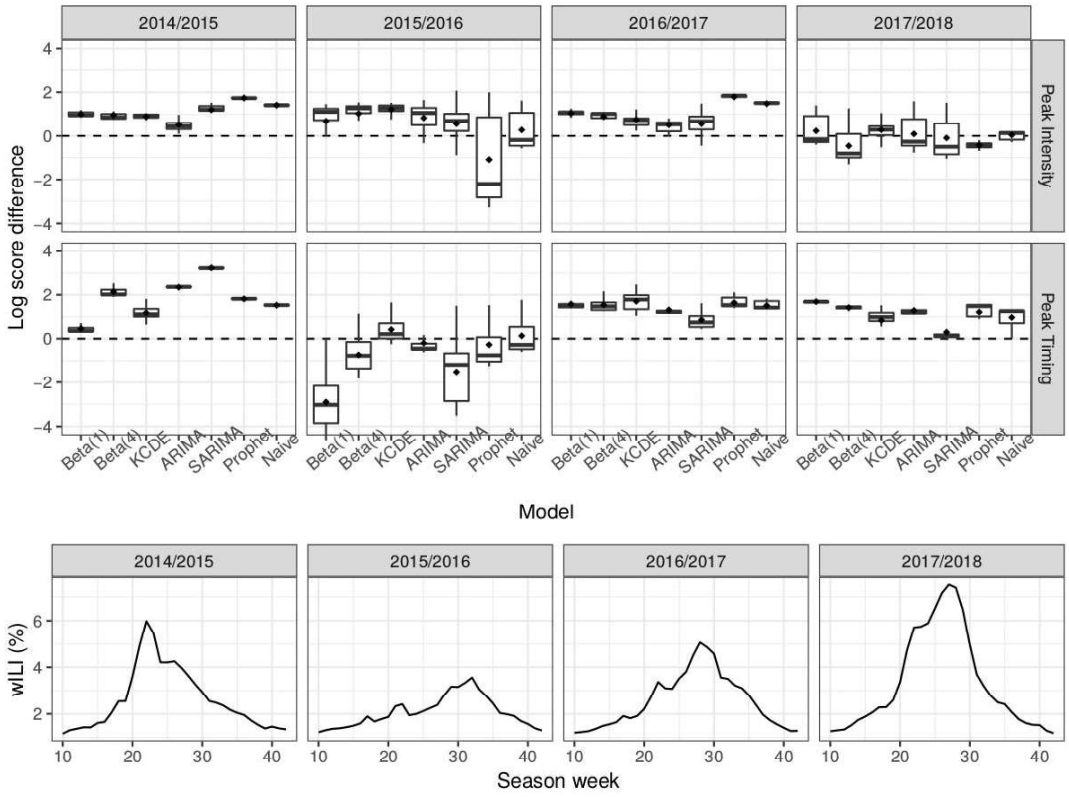
Others

Statistical Analysis of Infectious Disease Spread

Dr. Sebastian Meyer, Department of Medical Informatics, Biometry and Epidemiology

Epidemic models are used to analyse and forecast the spread of infectious diseases. Specialized regression methods for public health surveillance time series allow us, for example, to evaluate socio-demographic and environmental factors, and to generate probabilistic forecasts of disease spread. This project has started to extend the epidemiologist's statistical toolbox with respect to time series of proportions. All methods have been implemented in open-source software.

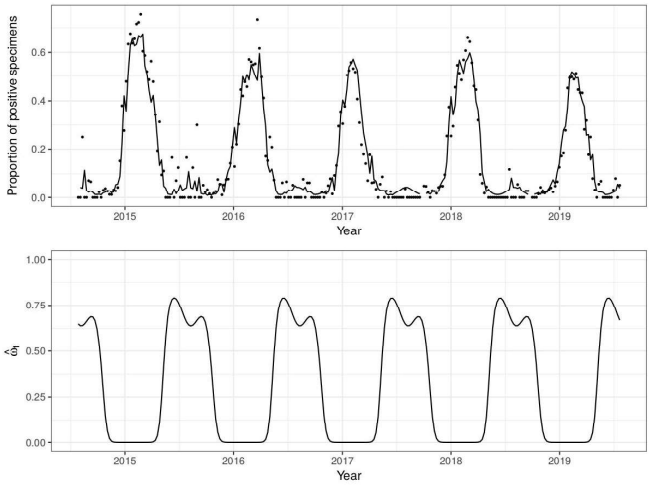
Infectious disease occurrence is frequently analyzed using a so-called endemic-epidemic modeling approach, where disease incidence is decomposed into an endemic and an epidemic (autoregressive) part. The endemic part typically represents seasonal variation and the epidemic part represents disease spread, i.e., the ability of infected persons (or animals) to trigger further cases during the infectious period. A well-known endemic-epidemic modeling framework is the so-called HHH model for (multivariate) time series of infectious disease counts from routine public health surveillance.



Each boxplot (top) summarizes the predictive performance of the given model compared to the equal bin reference approach. Positive values favor the model-based forecasts. The mean is indicated by dots. The bottom plot shows the epidemic curve for each season.

Dr. Meyer

In some applications, however, the proportion of infected individuals is of more interest than absolute counts. For example, the national weighted influenza-like illness (wILI) index is used by the Centers for Disease Control and Prevention (CDC) in the USA to measure flu activity. The wILI index is the proportion of outpatient visits for influenza-like illness, and has the total number of outpatient visits as its denominator. Forecasting the wILI proportion using an HHH model would require an extra model for these denominator counts. A more direct modeling approach for such proportion time series is desirable.



First row: proportion of positive specimens in the influenza laboratory surveillance data in Germany (points), and fitted value from a zero-inflated beta model (solid line). Second row: estimated mixture parameter (probability of the zero model).

We extended the HHH model for infectious disease proportions following a distributional regression approach with the beta distribution as the (conditional) response distribution. The proposed endemic-epidemic beta model uses a logit-link for the mean, a log-link for the precision and the logit transformation for the autoregressive part. The formulation of the mean is built upon simplicial geometry, which ensures symmetry of the beta model with respect to complementary proportions. Parameters of both the endemic and epidemic part can be interpreted in terms of odds ratios and are estimated using maximum likelihood inference via the R package “betareg”. Furthermore, we proposed a zero-or-one inflated beta model, for the case when the time series contains essential zeros or ones.

We investigated the predictive performance of the beta model for the wILI time series over 18 seasons. Compared with several other readily available forecasting tools, the beta model produced good short-term forecasts while having low complexity and short runtime. Real-time forecasting of the seasonal peak, however, should consider outputs of multiple models simultaneously, weighing their usefulness as the season progresses.

Future work will focus on multivariate versions of the beta model, taking into account regional flu activity or heterogeneity between age groups. Spatial power-law weights and discrete-time serial interval distributions can be borrowed from the multivariate HHH framework. Cooperations with the Robert Koch Institute have been initiated. Furthermore, a Master Thesis (Leonie Holtmann) showed a promising extension of the HHH model for zero-inflated infectious disease counts, which will be investigated further.

Contact:
Dr. Meyer
phone: +49 9131 85 22707
e-mail: seb.meyer@fau.de

Invited lectures

GEOMED 2019, 27 August 2019, Glasgow, Forecasting based on surveillance data

Publications during funding period

none

Newly started Projects

J76 16/10/2019 - 15/04/2022 Immunology and Infection

The role of itaconate-mediated metabolic reprogramming in osteoclasts

Dr. Katerina Kachler, Department of Medicine 3 – Rheumatology and Immunology

Dr. Kachler

The maintenance of healthy bone relies on a balance between bone formation by osteoblasts and bone resorption by osteoclasts. Recent findings indicate that the differentiation and function of osteoclasts can be regulated by certain changes in the cellular metabolism. Accordingly, we demonstrated that the metabolite itaconate inhibits osteoclastogenesis. The major aim of the presented project is the investigation of molecular mechanisms underlying this function of itaconate.

e-mail: katerina.kachler@uk-erlangen.de

J77 01/11/2019 - 30/04/2022 Immunology and Infection

Phenotypic and Transcriptional Characterization of Autoreactive B cells in Rheumatoid Arthritis

Dr. René Pfeifle, Department of Medicine 3 – Rheumatology and Immunology

Dr. Pfeifle

Rheumatoid arthritis is a common autoimmune disease. Anti-citrullinated-protein antibodies (ACPAs) have been identified as key players in RA . But a better understanding of the ACPA-producing B cells and of the transcriptional events in ACPA-specific B cells during the onset of RA is essential. Therefore, we plan to perform an in depth molecular characterization of the dynamic changes occurring inside the ACPA-specific B cell compartment during different phases of RA pathogenesis.

e-mail: rene.pfeifle@uk-erlangen.de

J78 01/10/2019 - 31/03/2022 Immunology and Infection

Role of GPX4-regulated ferroptosis in innate immunity to microbial infection

Dr. Ruder

Dr. Barbara Ruder,
Department of Medicine 1 – Gastroenterology, Pneumology and Endocrinology

Ferroptosis is a recently identified type of programed necrotic cell death which is tightly regulated by GPX4. In this project the exact role of GPX4 and ferroptosis in myeloid cells under steady state conditions and during Salmonella Typhimurium infection will be investigated. GPX4-regulated ferroptotic cell death might display a new therapeutic target for treatment of acute infections in human patients.

e-mail: barbara.ruder@uk-erlangen.de

J79 01/09/2019 - 28/02/2022 Immunology and Infection

Functional impact of PD-L1:PD-1-interactions on diet-induced obesity and dysbiosis

Dr. Schwartz

Dr. Christian Schwartz, Institute of Clinical Microbiology, Immunology and Hygiene

Obesity has become one of the leading global health concerns. Current research suggests a link between obesity, PD-L1 and the microbiome. This project will investigate the role of the microbiome on the function the regulation of adipose tissue T cell responses mediated by innate expression of PD-L1 in mice and humans. Using state-of-the-art RNA and 16S rRNA sequencing, flow cytometry and co-culture systems, we will reveal important mechanisms for the control of adipose tissue inflammation.

e-mail: christian.schwartz@uk-erlangen.de

Newly started Projects

J80 01/05/2020 - 31/10/2022 Oncology

Psen1 in colorectal cancer

Dr. Mahapatro

Dr. Mousumi Mahapatro, Department of Medicine 1 – Gastroenterology, Pneumology and Endocrinology

Presenilin (Psen1) has been demonstrated to be an important regulator of early-onset Alzheimer's disease. Interestingly, Psen1 is highly expressed in intestinal epithelium and plays an important role in mouse models of colorectal cancer. Preliminary results indicate that Psen1 regulates cancer stem cell population, proliferation and cell death. In this project we seek to identify signaling pathways executed by Psen1 and to provide new therapeutic alternatives in the treatment of cancer

e-mail: mousumi.mahapatro@uk-erlangen.de

J81 01/01/2020 - 30/06/2022 Medical Engineeringring

Web based Brain Tumor Image Classifier (WeB-TIC)

PD Dr. Jabari

PD Dr. Samir Jabari, Institute of Neuropathology

Low-grade epilepsy-associated brain tumours (LEAT) are rare entities with poor interobserver histopathology agreement. The WHO has established an integrated genotype- phenotype classification for most brain tumor entities, but not LEAT. Bioinformatical deep learning algorithms have proven success in extracting such genotype- phenotype information from histopathology slides. Our research proposal evolves around this innovative approach in order to provide diagnostically useful imaging biomarkers.

e-mail: samir.jabari@uk-erlangen.de

O-GlcNAcylation regulates osteoclastogenesis

Dr. Chen

Dr. Chih-Wei Chen, Department of Medicine 3 – Rheumatology and Immunology

We demonstrate that dynamic changes in the levels of O-GlcNAcylation are critically required for osteoclastogenesis. Inhibition of this dynamic regulation prevents osteoclast differentiation and ameliorates local and systemic bone loss in experimental arthritis. We now aim to further characterize the signaling pathways regulated by O-GlcNAcylation in inflammatory and non-inflammatory bone loss and to validate targeted modulation of O-GlcNAcylation as a therapeutic option for aberrant bone loss.

e-mail: chih-wei.chen@uk-erlangen.de

P016 01/02/2018 - 31/01/2019 Others

Laboratory detection of ultrasound microbubbles

Dr. Ferdinand Knieling, Department of Pediatric and Adolescent Medicine

Contrast Enhanced Ultrasound (CEUS) is an established diagnostic procedure in adults. It is highly accurate, easy to use, free of ionizing radiation and does not require anesthesia / sedation in children. The aim of this project is the establishment of a laboratory methodology for the detection of the used microbubbles. In addition to analytical parameters, the preanalytical conditions are investigated.

e-mail: ferdinand.knieling@uk-erlangen.de

P018 01/03/2018 - 28/02/2019 Oncology

XCR1 as a marker for human crosspresenting DCs

Dr. Lukas Heger, Department of Dermatology

DCs have an important role in der regulation of immune responses. Recent studies in mice suggest that crosspresentation is a specialized function of XCR1+ cDC1. Also in humans different DC subsets can be differentiated based on surface markers, which show transcriptional homologies to murine DC subsets. Therefore, this proposal aims to analyze whether XCR1 is a marker for human crosspresenting DCs and whether these polarize naïve T cells mainly into Th1 cells due to enhanced IL-12 secretion.

e-mail: lukas.heger@uk-erlangen.de

P019 01/06/2018 - 30/04/2019 Others

The involvement of the enteric nervous system in the immunopathogenesis of multiple sclerosis

Prof. Dr. Stefanie Kürten, Institute of Anatomy and Cell Biology

The proposal evolves around the central aim to provide a precise morphological analysis of enteric nervous system (ENS) pathology in MP4-induced experimental autoimmune encephalomyelitis (EAE) as a mouse model of multiple sclerosis (MS). It can be further subdivided into two main objectives. While objective 1 will determine the extent of submucous plexus pathology, objective 2 aims to identify the exact neuronal target population(s) of ENS-reactive autoantibodies in MP4-induced EAE.

e-mail: stefanie.kuerten@fau.de

P020 24/01/2018 - 23/01/2019 Oncology

Localisation of the EMT-transcription factor ZEB1

Dr. Rebecca Eccles, Chair of Experimental Medicine I

ZEB1 is a major driver of cancer metastasis, the leading cause of cancer-associated death, as it couples the activation of cellular motility with stemness and survival properties. As a transcription factor ZEB1 is normally found in the nucleus. However, we have observed cytosolic ZEB1 in a variety of cancer types, and the presence of this form seems to correlate with better patient prognosis. We therefore plan to explore the role cytosolic ZEB1 plays in cancer and metastasis.

e-mail: rebecca.eccles@fau.de

P021 01/06/2018 - 30/04/2019 Immunology and Infection

Bone strength in rheumatic finger joints

Dr. Arnd Kleyer, Department of Medicine 3 – Rheumatology and Immunology

The aim of this study was to develop a FEA model for the Metacarpo-phalangeal (MCP) joints imaged with HR-pQCT to understand the impact of changes in bone density and microarchitecture due to inflammation on bone strength of the MCP head. Segmented MCP heads were divided into trabecular and cortical regions and modeled by FEA (Faim v8.0, Numerics88 Solutions Ltd., Canada). Failure load was based on 2% critical volume at 0.007 strain. To transfer the force of 100 N into the joint, a polymethylmethacrylat (PMMA) cap was added at the distal end of the MCP head. Bone stiffness ranged from 4.4 - 6.8 kN/mm and failure load from 540 N to 780 N. Displacement decreases in the proximal direction.

e-mail: arnd.kleyer@uk-erlangen.de

P022 01/08/2018 - 31/07/2019 Others

Vascularization and bone formation

Dr. Dominik Steiner, Department of Plastic and Hand Surgery

The broad use of bioartificial bone tissue is limited by the insufficient integration into the host vasculature. One strategy to improve vascularization is the generation of an arteriovenous fistula (AV loop). The goal of this project is to establish a cell-loaded hydrogel matrix for the reconstruction of large bone defects. Human ADSCs and HUVECs will be encapsulated in a hydrogel matrix, vascularization will be performed by means of an AV loop and BMP-2 will enhance bone formation.

e-mail: dominik.steiner@uk-erlangen.de

P023 01/06/2018 - 31/05/2019 Immunology and Infection

DPP4 - a molecular target in fibrosis

Dr. Alina Soare, Department of Medicine 3 – Rheumatology and Immunology

Fibrotic diseases can be considered as a consequence of persistent, exaggerated and uncontrolled tissue repair processes. Systemic sclerosis (SSc) is associated with a high mortality and effective anti-fibrotic therapies are still lacking. Dipeptidyl-peptidase-4 (DPP4) has been recently shown to identify a distinct dermal lineage of fibroblasts involved in scar formation. We have demonstrated that DPP4-positive cells are increased not only in experimental fibrosis, but also in skin biopsies from SSc patients as compared to healthy volunteers. DPP4-inhibitors showed potent anti-fibrotic effects in bleomycin-induced skin fibrosis. However, the specific role of DPP4-positive fibroblasts in fibrotic diseases is unknown. We aim in this study to characterize the phenotype of DPP4-positive fibroblasts and assess the potential pro-fibrotic properties of DPP4-positive fibroblasts. Further we plan to test DPP4-inhibitors in mouse models of fibrotic diseases resembling, different subtypes and stages of other fibrotic diseases such as chronic graft-versus-host disease mouse model and models of pulmonary fibrosis. These results may have direct clinical implications as DPP4-inhibitors are already in clinical use for diabetes.

e-mail: alina.soare@uk-erlangen.de

P024 01/10/2018 - 31/03/2019 Immunology and Infection

The contribution of butyrophilins in the pathogenesis of Rheumatoid Arthritis

Dr. Kerstin Sarter-Zaiss, Department of Medicine 3 – Rheumatology and Immunology

The aim of this project is to analyze the role of the co-stimulatory molecule Btn2a2 in the development and / or pathology of RA and to lay the foundations for future research to understand the underlying mechanisms of action of Btn2a2 during the dissolution of inflammation decrypt.

e-mail: kerstin.sarter-zaiss@uk-erlangen.de

P025 01/08/2018 - 31/07/2019 Others

Analysis of exosomal biomarkers for CRSwNP

Dr. Sarina Müller, Department of Otorhinolaryngology - Head and Neck Surgery

Exosomes are spherical 30-150nm vesicles which are secreted by virtually all cell types in virtually all body fluids. For this reason, exosomal proteins are an innovative and non-invasive way for the analysis of biomarkers. We could identify Pappalysin-A (PAPP-A) in own preliminary studies as a promising biomarker for chronic rhinosinusitis with nasal polyps (CRSwNP). The objective of this project is the detailed analysis of Pappalysin-A and its evaluation as a potential biomarker for CRSwNP.

e-mail: sarina.mueller@uk-erlangen.de

P026 15/11/2018 - 14/11/2019 Neurosciences

On myelination processes in the cuprizone model

Prof. Dr. Frederik Laun, Institute of Radiology

The aim of this work is gaining knowledge about the ability of quantitative susceptibility mapping (QSM) to monitor de- and remyelination processes. QSM is a rather novel magnetic resonance imaging (MRI) approach that reveals the magnetic susceptibility of tissue, which, e.g., indicates the presence of myelin. Cuprizone mouse models shall be used, which allow monitoring de- and remyelination. Cuprizone is a copper chelator that induces a demyelination process.

e-mail: frederik.laun@uk-erlangen.de

P027 01/07/2018 - 31/12/2019 Oncology

IFN-gamma and vasculature in CRC

Dr. Nathalie Britzen-Laurent, Department of Surgery

The microenvironment is critical for tumor growth and regulates both angiogenesis and the anti-tumor immune response. We have previously shown that IFN-gamma, a mediator of the anti-tumor immune response can also influence the vasculature by inhibiting angiogenesis and inducing vessel permeability in vitro and in vivo. Here, we will investigate the vascular effects of IFN-gamma and their influence on tumor development using a chemically-induced colon carcinogenesis mouse model.

e-mail: nathalie.britzen-laurent@uk-erlangen.de

P028 01/10/2018 - 31/09/2019 Neurosciences

3D organoid models for analysis of SOX11-CSS

Dr. Sören Turan, Institute of Biochemistry

Coffin-Siris syndrom (CSS) is a neurodevelopmental disorder with cardinal features of intellectual disability and microcephaly. Mutations of transcription factor SOX11 were described as a genetic cause of CSS. In order to allow a better understanding of the pathophysiological consequences of SOX11 haploinsufficiency during human cortex development, this proposal aims to generate and analyse a SOX11+/- cerebral organoid model.

e-mail: soeren.turan@fau.de

P029 01/11/2018 - 31/10/2019 Oncology

Combined DNA-/RNA-transfection of T cells

Dr. Ugur Uslu, Department of Dermatology

Tumor cells can by-pass T-cell recognition, which is a challenge in adoptive T-cell therapy. To increase the pressure on the tumor, we want to generate T cells expressing two additional tumor-specific receptors by combining stable DNA- and transient RNA-based receptor transfer. The latter of the two receptors shall have a “boost” effect in the first days of therapy by induction of direct tumor-cell killing and T-cell proliferation, leading to an effective tumor cell lysis.

e-mail: ugur.uslu@uk-erlangen.de

P030 01/01/2019 - 31/12/2019 Neuroschiences, Others

Ultrashort Echo Time MRI of Myelin at 7 T

Prof. Dr. Armin Nagel, Institute of Radiology

The aim of this project is to implement and to evaluate an ultra-short echo time (UTE) pulse sequence for clinical and preclinical research at 7 Tesla, which enables non-invasive mapping of the myelin content. Image artifacts - that are common in UTE imaging - will be analyzed and corrected. The newly implemented UTE imaging technique will be applied in two proof-of-concept studies, including a mouse model of multiple sclerosis and multiple sclerosis patients.

e-mail: armin.nagel@uk-erlangen.de

P031 16/12/2018 - 15/06/2019 Neurosciences

Zwicker tone as a model for acute tinnitus

Dr. Achim Schilling, Department of Oto-Rhino-Laryngology - Head and Neck Surgery

The main goal of the here proposed project is the identification of the cortical representation of a Zwicker tone as a model for tinnitus. Thus, we plan to carry out MEG and EEG measurements in cooperation with PD Dr. Rampp, to compare the neuronal representation of silence, pure-tones and Zwicker tones. As we expect neuronal mechanisms within the dorsal cochlear nucleus as cause for acute tinnitus, we consider the cortical representation of Zwicker tones and pure tones to be similar.

e-mail: achim.schilling@uk-erlangen.de

P032 15/07/2018 - 14/07/2019 Immunology and Infection

In vivo imaging of inflammation / bone remodeling

Dr. Christine Schauer, Department of Medicine 3 – Rheumatology and Immunology

Abnormal neo-ossification in humans with SpA and gout are common, but there are only few suitable animal models. Conventional methods require a high number of animals or can only insufficiently distinguish between inflammatory edematous swelling and/or increased bone thickness. The aim of this application is to establish a mouse model of gouty enthesitis in vivo by means of a non-invasive combination of MRI and PET/CT, in which live animals can longitudinally be examined.

e-mail: christine.schauer@uk-erlangen.de

P033 26/03/2019 - 25/03/2020 Others

YB-1 in molecular regulation of chondrogenesis

Dr. Ulrike Rottensteiner-Brandl, Institute of Biochemistry

YB-1 is expressed in chondrocytes during late embryonic stages and after birth, suggesting a crucial role of YB-1 in chondrogenesis. Little is known about signaling cascades linked to YB-1 mediating its action. The goal of this study is to investigate YB-1-dependent pathways during chondrogenesis. Particular interest will be paid to MIA-dependent signaling (upstream) and target genes involved in transition to hypertrophy and dedifferentiation (downstream).

e-mail: ulrike.rottensteiner@fau.de

P034 16/01/2019 - 15/01/2020 Oncology

Myeloid ZEB1 in colorectal cancer

Dr. Harald Schuhwerk, Chair of Experimental Medicine I

In colorectal cancer (CRC), the transcription factor ZEB1 is upregulated in tumor cells and tumor-associated macrophages. As only its tumor-promoting role in tumor cells is known, we are analyzing myeloid-specific ZEB1 knockout mice. Our preliminary data suggest that ZEB1 plays a role in macrophage polarization, intestinal inflammation and CRC growth. Here, we will explore novel functions of ZEB1 in immune homeostasis, macrophage plasticity, immune-modulation in CRC and colitis-associated CRC.

e-mail: harald.schuhwerk@fau.de

P035 07/01/2019 - 06/01/2020 Neurosciences

Role of FBXO11 in intellectual disability

Dr. Anne Gregor, Institute of Human Genetics

Recently we identified de novo variants in FBXO11, encoding a subunit of an E3-ubiquitin ligase complex, as causative for a neurodevelopmental disorder (NDD). The goal of this grant is to characterize the role of FBXO11 in NDDs. With the model organism Drosophila melanogaster anatomical studies of synapses and behavioral assays will be performed. Additionally effects of patient mutations will be tested in cell-based assays and target proteins of FBXO11 will be identified using AP-MS.

e-mail: anne.gregor@uk-erlangen.de

P036 01/04/2019 - 31/03/2020 Others

Physicians' opinions on continuous sedation

Dr. Maria Heckel, Division of Palliative Medicine

Physicians' practice and opinions regarding continuous sedation until death are to be collected internationally. The German subproject aims to gain a comprehensive overview of the opinions of German palliative physicians by online survey and to link them to their professional background and experiences. The crosscultural comparison might contribute to an internationally binding definition and a more uniform treatment practice.

e-mail: maria.heckel@uk-erlangen.de

P037 01/07/2019 - 30/06/2020 Neurosciences

Prognostication in intracerebral hemorrhage

Dr. Jochen Sembill, Department of Neurology

Prognostication in intracerebral hemorrhage (ICH) is biased by self-fulfilling prophecy. We will 1) validate the max-ICH Score, pooling patient data from i) single-center study from Massachusetts General Hospital (Harvard), ii) single-center UKER study, iii) multicenter RETRACE study. We will 2) conduct a prospective multicentre study with randomized controlled prognostic score usage to evaluate physician's prognostic variability & accuracy, optimal prognostic timing, improved outcome measures.

e-mail: jochen.sembill@uk-erlangen.de

P038 01/04/2019 - 31/03/2020 Oncology

Molecular markers in stage T1 bladder cancer

Dr. Danijel Sikic, Department of Urology and Paediatric Urology

Previous studies demonstrated a prognostic relevance of several molecular markers in stage T1 bladder cancer. These might optimize risk stratification and decision making with regard to immediate cystectomy or bladder sparing approach. However, these findings have not been validated yet.

The goal of the current study is to validate the association of the mRNA expression of these molecular markers with clinical and survival data in a new cohort consisting of stage T1 bladder cancer.

e-mail: danijel.sikic@uk-erlangen.de

P039 01/06/2019 - 31/05/2020 Immunology and Infection

Bone characterization in early RA autoimmunity

Dr. David Simon, Department of Medicine 3 – Rheumatology and Immunology

To better understand the influence of the early phase of autoimmunity of rheumatoid arthritis (RA) on joint structure, longitudinal observations of pre-RA patients are necessary. High-resolution CT is used to investigate how bone density and structure and biomechanical properties of pre-RA patients develop over time, what influence different biomarker profiles have and what bone characteristics patients developing clinical RA have.

e-mail: david.simon@uk-erlangen.de

P040 01/09/2019 - 31/08/2020 Neurosciences

FoxO-dependent mitophagy in stem cell function

Dr. Iris Schäffner, Institute of Biochemistry

Mitochondrial function is crucial for maintenance of the adult neural stem/progenitor cell (NSPC) pool. I found that loss of FoxO transcription factors leads to hyperproliferation and depletion of NSPCs and impairs autophagy-lysosome pathway activity. Moreover, loss of FoxOs is associated with mitochondrial dysfunction. I propose to investigate mitochondria as targets of FoxO-dependent autophago-lysosomal degradation, to establish a FoxO-mitophagy axis in the control of adult NSPC function.

e-mail: iris.schaeffner@fau.de

P041 01/09/2019 - 31/08/2020 Renal and Vascular Research

Polyploid cardiomyocytes for cardiac repair

Dr. Maria Leone, Department of Nephropathology

Humans are incapable to regenerate their heart. Cardiac injury results in cardiomyocyte loss due to hypoxia and a changed mechanical micro-environment. Here we propose to determine the potential of polyploid cardiomyocytes, the majority in the adult heart, to contribute to heart repair. We propose to clarify if polyploid cardiomyocytes can be induced to proliferate or whether diploid and polyploid cardiomyocytes differ in regards to stress resistance, cell size, and mechanical properties.

e-mail: marina.leone@uk-erlangen.de

P042 01/09/2019 - 31/08/2020 Oncology

Immunology of NMSC of the head and neck

Dr. Dr. Gesche Frohwitter, Department of Oral and Cranio-Maxillofacial Surgery

The facial skin is most frequently affected by non-melanoma skin cancer (NMSC). However, the immunological profile of these tumors is still poorly understood. The anticipated ELAN project aims to address this problem by immunohistochemical investigations and may anticipate the establishment of an immunoscore which supplements the TNM classification in prognostic information and therapeutic decision making.

e-mail: gesche.frohwitter@uk-erlangen.de

P043 01/07/2019 - 30/06/2020 Oncology

The autotaxin-LPA axis in breast cancer

Dr. Annika Kengelbach-Weigand, Department of Plastic and Hand Surgery

Breast cancer is the most common cancer in women worldwide. It is hypothesized that in a vicious cycle autotaxin (ATX) secreted by fat tissue influences breast cancer cells in behavior and leads to secretion of inflammatory cytokines which in turn stimulate ATX secretion of fat tissue. Radiotherapy could lead to an amplification of this effect. It is the aim of this study to evaluate the significance of the ATX/LPA-axis and the effect of radiotherapy in different breast cancer subtypes.

e-mail: annika.kengelbach-weigand@uk-erlangen.de

P044 16/08/2019 - 15/08/2020 Immunology and Infection

HIF-1a in IgA class switching

Dr. Xianyi Meng, Department of Medicine 3 – Rheumatology and Immunology

Germinal center (GC) has been described to contain hypoxic regions linked to B cell class switching. In this project, we will delineate molecular mechanism between HIF-1a-dependent glycolysis and epigenetic modification on IgA class switching region. By studying the IgA response following the C. rodentium infection, we aim to identify the link between the HIF-1a-dependent glycolytic metabolic shift and IgA class switching during microbial infection.

e-mail: xianyi.meng@uk-erlangen.de

P045 16/06/2019 - 15/06/2020 Immunology and Infection

HSV-1 modulates the IL-6 signaling pathway in mDCs

Dr. Linda Grosche, Division of Immune Modulation

The focus of the present project is the investigation of Herpes simplex virus type-1 (HSV-1)-mediated modulations of the IL-6 signaling pathway in mature dendritic cells (mDCs). In particular, the underlying molecular mechanisms of reduced IL-6Rα, gp130 and STAT3 expression will be analyzed on directly-infected versus uninfected bystander mDCs. Moreover, we will elucidate whether non-infectious L-particles, released from HSV-1-infected cells, are essential/sufficient to induce these modulations.

e-mail: linda.grosche@uk-erlangen.de

P046 01/11/2019 - 31/10/2020 Oncology

Magnetic Drug Targeting for head and neck cancer

Dr. Matthias Balk, Department of Oto-Rhino-Laryngology - Head and Neck Surgery

The main goal in this project is to evaluate the effect of supramagnetic iron oxide nanoparticles for the treatment of head and neck cancer. At first, we will investigate the effects of specific supramagnetic iron oxide nanoparticles on head and neck cancer cell lines. Subsequently, the oncologic potential of supramagnetic iron oxide nanoparticles loaded with chemotheraputics will be analyzed on the various generated cell lines.

e-mail: matthias.balk@uk-erlangen.de

P047 16/06/2019 - 15/06/2020 Neurosciences

Synaptic plasticity in the human hippocampus

Dr. Anna Maslarova, Department of Neurosurgery

Synaptic plasticity refers to activity-dependent strengthening or weakening of synaptic transmission and underlies memory formation. It can be investigated in-vitro by repetitive network stimulation. In patients with temporal lobe epilepsy, affecting the memory-related hippocampal formation, deficits in memory can occur. We aim to investigate the link between synaptic plasticity and memory impairment by in-vitro field potential recordings from human hippocampus removed during epilepsy surgery.

e-mail: anna.maslarova@uk-erlangen.de

P048 16/05/2019 - 15/03/2020 Immunology and Infection

Role of Collagen IV during retrovirus transmission

Dr. Andrea Thoma-Kreß, Institute of Clinical and Molecular Virology

The oncogenic retrovirus Human T-cell leukemia virus type 1 transmits via cell-cell contacts and viral biofilms seem to constitute a major route of virus transmission. In viral biofilms, extracellular concentrated viral particles are embedded in cocoon-like structures containing collagens (COL) of unknown composition. Here, we hypothesize that the viral transactivator Tax-1 selectively induces expression of COL4 and that COL4 is important for biofilm formation and HTLV-1 transmission.

e-mail: andrea.thoma-kress@uk-erlangen.de

P049 16/10/2019 - 15/10/2020 Immunology and Infection

Inflammation from skin to joint

Dr. Maria Gabriella Raimondo, Department of Medicine 3 – Rheumatology and Immunology

To date, it is still obscure why in some patients with psoriasis the autoimmune process is restrained to the skin, whereas in other it extends to the joints. We will take advantage of models resembling psoriatic arthritis, with the aim of studying the joint involvement secondary to skin inflammation. The understanding and characterization of the underlying mechanisms involved in the “skin-joint axis” is pivotal for a better comprehension of the link between physical barriers and autoimmunity

e-mail: mariagabriella.raimondo@uk-erlangen.de

P050 01/01/2020 - 31/12/2020 Immunology and Infection

Vascular, renal parameters in living kidney donors

Dr. Dennis Kannenkeril, Department of Medicine 4 – Nephrology and Hypertension

This study is a single-centre clinical study with 25 potential kidney donors. This is an exploratory and non-confirmatory study, in which we analyse different vascular and renal parameters in potential kidney donors before and after donation. Moreover, a kidney biopsy sample is obtained during donation. Our hypothesis is that histological scoring of renal damage (total renal chronicity scores) correlates with vascular parameters indicating increased stiffness. The primary vascular parameter is wall to lumen ratio of retinal arterioles. Moreover we hypothesize that vascular parameters predicts 24-hour blood pressure and renal outcome (eGFR, albuminuria) one year after donation.

e-mail: dennis.kannenkeril@uk-erlangen.de

P051 01/01/2020 - 31/12/2020 Oncology

T follicular helper cells, transplantation

Dr. Silvia Spörl, Department of Medicine 5 – Hematology and Oncology

T cells play a major role in complications after allogeneic stem cell transplantation (allo-HSCT). In this projekt we will examine characteristics of T follicular helper cells in patients at different timepoints before and after allo-HSCT and we will correlate them with severe complications (e.g. EBV reactivation or GvHD). With this study, we aim to get more insight in phenotypes and function of Tfh- especially in the context of allo-HSCT.

e-mail: silvia.spoerl@uk-erlangen.de

P052 01/10/2019 - 30/09/2020 Oncology

Treatment Response in EwS by PET/CT and ctDNA

Dr. Christian Schmidkonz, Department of Nuclear Medicine

18F-FDG PET/CT is as promising tool for determining treatment response in Ewing Sarcoma (EwS). Standardized uptake values as marker for metabolic tumor activity can be determined in serial scans to determine response to therapy. EwS is characetrised by circulating tumor DNA (ctDNA) that can be quantified from patients' plasma. We intend to use 18F-FDG-PET/CT and ctDNA to determine treatment response in 20 children and adolescents suffering from EwS.

e-mail: christian.schmidkonz@uk-erlangen.de

P053 01/10/2019 - 31/03/2020 Immunology and Infection

The influence of serotonin on antigen presentation

Dr. Sascha Kretschmann, Department of Medicine 5 – Hematology and Oncology

Non-classical HLA class II molecule HLA-DO largely influences the presented peptide repertoire in HLA class II. HLA-DO expression was shown to play a role in type 1 diabetes, in the generation of neutralizing antibodies to viral infections and in immune responses after allogeneic stem cell transplantation. However, the regulation of HLA-DO is distinct from other class II molecules and remains elusive. We here hypothesize that serotonin receptor signaling plays a role in the regulation of HLA-DO.

e-mail: sascha.kretschmann@uk-erlangen.de

P054 01/10/2019 - 30/09/2020 Neurosciences

Agrin and the neuromuscular junction in ALS

Dr. Florian Krach, Department of Stem Cell Biology

ALS is a neurological disorder molecularly manifesting in pathological aggregation of the splicing regulator TDP-43 and altered splicing in neurons (N). I aim to investigate an identified exon inclusion event in the neuromuscular junction inducer Agrin in a stem cell-based model of ALS. I use a microfluidics co-culture system of Ns and myoblasts. Additionally, in the same system, I analyze the effects of exon exclusion as seen in ALS post mortem tissue.

e-mail: florian.krach@uk-erlangen.de

P055 28/10/2019 - 27/10/2020 Others

MSOT-imaging in spinal muscular atrophy

Dr. Adrian Regensburger, Department of Pediatrics and Adolescent Medicine

New non-invasive imaging biomarkers for childhood muscular diseases are not yet established. Using multispectral optoacoustic tomography (MSOT), we were already able to detect collagen as a potential biomarker for disease progression monitoring of patients with Duchenne muscular dystrophy. In this experimentell study, we will investigate, which specific optoacoustic spectrum could serve as a biomarker in patients with spinal muscular atrophy.

e-mail: adrian.regensburger@uk-erlangen.de

P056 01/04/2020 - 31/03/2021 Immunology and Infection

Host-microbial interaction in the Liver

PD Dr. Dr. Claudia Günther, Department of Medicine 1 - Gastroenterology, Pneumology and Endocrinology

Liver biology and liver diseases are difficult to study using current in vitro models. We developed a new method to isolate and expand self-renewing liver organoids from the embryonic liver. Within this project we aim to understand how microbiome-associated signaling pathways influence maturation, injury and regeneration of the liver by using these organoids. Thus this project will provide new insights in the critical role of liver-gut communication and potentially hepatic disease development.

e-mail: c.guenther@uk-erlangen.de

P057 01/11/2019 - 31/10/2020 Immunology and Infection

Intravital microcopy in the AV-loop model

Dr. Maximilian Hessenauer, Department of Plastic and Hand Surgery

Tissue engineering in reconstructive surgery seeks to generate bioartifical tissue substitutes. The AV-loop allows generation of axially vascularized tissue. Cellular mechanisms of this process are largely unclear. Therefor the proposed project aims to evaluate leukocyte mediated processes in this context. Using intravital microscopy, the role of different leukocyte subsets is going to be evaluated. This is aimed to provide novel understanding of these processes for therapeutic application.

e-mail: maximilian.hessenauer@uk-erlangen.de

P058 12 months Oncology

Wnt inhibitory peptide

Dr. Dominic Bernkopf, Chair of Experimental Medicine II - Molecular Oncology

A synthetic peptide inhibits Wnt/ß-catenin signalling and growth of colorectal cancer cells by augmenting conductin-mediated ß-catenin degradation. Here, we want to improve peptide activity by optimising its functional and its cell permeability-providing parts. Then, cell penetration kinetics, cellular distribution and stability will be characterised, and the optimised peptide will be functionally compared to the old version to verify improvement of our peptide towards therapeutic applicability.

e-mail: dominic.bernkopf@fau.de

P059 01/03/2020 - 28/02/2021 Neurosciences

Regional neuroinflammation in RA

Dr. Patrick Süß, Department of Molecular Neurology

Rheumatoid arthritis (RA) is linked to neuropsychiatric comorbidity like depression due to inflammatory brain involvement. This project aims to investigate the influence of chronic peripheral inflammation on the blood brain-barrier and macrophages in different brain regions in an RA mouse model and human post mortem tissue. Thereby, local factors promoting inflammatory susceptibility or resilience may be identified as therapeutic targets for the CNS involvement in RA.

e-mail: patrick.suess@uk-erlangen.de

P060 12 months Oncology

ERVs in the tumorigenesis of MIBC

Dr. Markus Eckstein, Department of Pathology

To investigate if high amounts of tumor cell dsRNA derived from ERVs regulate the tumor immune microenvironment by activating the 'viral alarm' or IFN response leading to high anti-tumor lymphocyte infiltration including establishment of tertiary lymphoid structures as well as adaptive responses (immune checkpoints, negative regulatory immune cells and ECM production) already in early precursor stages of muscle-invasive bladder cancer and how they might evade the immunosurveillance.

e-mail: markus.eckstein@uk-erlangen.de

Modified proteins in the gut break the tolerance and initiate autoimmune arthritis

Prof. Dr. Mario Zaiss, Department of Medicine 3 – Rheumatology and Immunology

In RA the degree of inflammation and autoantibody positivity are important initiators of bone destruction. Interestingly, among IBD patients with chronic gut inflammations about 45% were positive for at least one arthritis antibody. However, despite the reported higher incidence of bone destruction in IBD patients, it remains elusive whether and how local gut antibody production and their different posttranslational modifications during gut inflammations directly contribute to RA.

e-mail: mario.zaiss@uk-erlangen.de

Index of Names

F

G

H

N

P

R

S

T

U

V

W

Z