Department of Biomedicine
Report 2014–2016

Departement
Biomedizin
Basel


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Department of Biomedicine
Report 2014–2016

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136 Focal Area **Immunology**

Preface

Departement
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The Department of Biomedicine (DBM) unites the laboratory-based research of the faculty of medicine of the University of Basel. In the DBM, the laboratories of the former "pre-clinical institutes" as well as the clinical divisions of the University Hospitals are brought together under a common leadership with the goal to focus their efforts and to strive for excellence in biomedical research. In the 16 years since the department was founded, we enjoyed continuous growth and flourishing of our research. By providing a bridge between basic science and clinical medicine, the DBM is an important component in the University of Basel's strategic plan for the Life Sciences and a key player in the national "Swiss Personalized Health Initative" (SPHN). The DBM concentrates on research in four focal areas: Oncology, Immunology, Neurobiology, and Stem Cells/Regenerative Medicine.

DBM's research groups obtain a large proportion of their research funds from competitive grants by national foundations, the EU and other countries. More than 50 % of the positions are funded by third parties. The DBM has attracted individual grants as well as synergy grants from the European Research Council (ERC), the Swiss Initiative in Systems Biology (SystemsX.ch) and the Swiss National Science Foundation (SNSF).

This report summarizes the activities of over 60 DBM research groups during the period of 2014–2016. The reports are grouped thematically according to the four focal areas. Each research group has selected their most relevant publications from this period. A complete list of all publications can be found in the annex of this report. The DBM and our research groups are regularly evaluated by the Scientific Advisory Board that consists of eight internationally recognized experts. During their yearly visits, the Advisory Board members evaluate and make recommendations on how to improve the organization of the department. They also provide an important basis for decisions, including promotions and changes in future directions. Key to the success of the DBM has been the enthusiasm of our scientists and clinicians from over 40 countries to communicate and to perform inter- and trans-disciplinary work resulting in benchmark biomedical research. The research is supported by a growing number of Core Facilities. While some Core Facilities are for the DBM only, others are joint ventures between our department with the Biozentrum (Faculty of Natural Sciences) and also the D-BSSE Institute of the ETH Zürich in Basel. The access to these key technologies is of immeasurable value to us.

Since January 2014, the DBM obtained new laboratories on the 2nd floor of the DBM-Hebelstrasse, where the former medical library has been converted to lab space. This much-needed expansion has allowed recruitment of additional junior faculty supported by SNF. A major milestone is the project for a new DBM building, which will replace the old Biozentrum located at Klingelbergstrasse 70. The new DBM building is scheduled to be inaugurated in 2023 and will unite all DBM research groups in a single location situated in close vicinity of the new Biozentrum and the new D-BSSE of the ETH. This new campus will provide an excellent basis for expanding collaborative research between clinical and basic science at the highest competitive level.

The DBM is committed to the highest quality and innovation in research. This report portraits the scientific excellence and enthusiasm of our research groups. I wish you pleasant reading.

Prof. Dr. Radek Skoda
Head of the Department of Biomedicine

International PhD Program in Biomedicine

Dissertations 2014–2016

As of 2015 all PhD students of the DBM are part of the International PhD program in Biomedicine. Within this interdisciplinary environment more than 120 PhD students from 28 countries are trained on the postgraduate level aiming the PhD degree from the Faculty of Science. Our program provides in-depth experimental competences and scientific knowledge in a wide range of disciplines of basic and clinical research. Our interdisciplinary PhD Program is associated with the PhD Program in Molecular and Cellular Biology offered by the Biozentrum. Every PhD student at the DBM receive theoretical and practical training, and conduct a research project under the supervision of a DBM research group leader recognized by the Faculty of Science, and monitored by a PhD thesis advisory committee. A rich program of courses, lectures, workshops and conferences organized by the Department of Biomedicine and the Biozentrum are offered to the PhD students. PhD fellows attend yearly scientific meetings (DBM PhD scientific retreat) and have the opportunity to organize seminar series and career guidance events.

The DBM PhD Club creates a department-wide student's club organizing various activities for the PhD students at the DBM with the aim to establish a basis for enhanced scientific networking and exchanges between students of the DBM. They organized the DBM PhD Scientific Retreat which takes place in general in the spring semester as it did in Hasliberg (2014), Schwarzsee (2015) and Emmeten (2016), and is an excellent platform to form new collaborations in form of knowledge, methodological and technical transfer. In the fall semester they organize the Career Day inviting speakers talking about future career development. This can be either for a scientific track – what kind of financial funding of early and late postdoc stipendium are provided by the Swiss National Science Foundation – or opportunities in Private Industry.

The PhD Program of the DBM aims to provide a scientific as well as an educational platform for PhD students allowing them to shape on one side their individual scientific career but on the other side to establish their first scientific network.

Group Bernhard Bettler

Lisa Adelfinger (2014)
Modulation of GABAB receptor signaling by associated proteins and phosphorylation

David Berner (2016)
GABAB receptor-associated KCTD proteins as molecular linkers to downstream signaling complexes

Group Josef Bischofberger

Stefanie Heigele (2015)
Bidirectional GABAergic control of AP firing in newly-generated young granule cells of the adult hippocampus

Group Daniel Bodmer

Marijana Sekulic (2015)
Characterization of the excitation-contraction coupling in extraocular muscles

Group Claudia Cavelti-Weder

Angela Bosch (2014)
The role of the transcription factor Foxn1 in Thymus organogenesis and maintenance

Group Gerhard Christofori

Ruben Bill (2015)
Mechanisms of action and resistance to novel targeted therapies in preclinical

Maren Diepenbruck (2015)
Transcriptional and posttranscriptional control of epithelial-mesenchymal transition

Laura Estelle Pisarsky (2016)
Angiogenesis: from tumor initiation to therapeutic resistance

Dana Ronen (2016)
Lose Cancer – Gain Fat: Forced Differentiation of EMT-derived Cancer Cells to Inhibit Cancer Metastasis

Group Christian De Geyter
Xiaoli Shen (2015)
Role of HECTD1 in regulating adhesion dynamics during cell movement

Group Marc Donath
Erez Dror (2015)
Physiological synergy between IL-1β and insulin on glucose disposal and macrophage activity

Thierry Nordmann (2016)
Identification, functional characterization and role of islet-associated immune cells in the pathogenesis of type 2 diabetes

Constanze Thienel (2015)
Sirtuin 1 and angiotensin II as inflammatory modulators in the development of diabetes

Shuyang Traub-Xu (2014)
Role of Alpha Cell-Derived Glucagon-Related Peptides on Glucose Metabolism

Group Daniela Finke
Simone Neu (2015)
Conditioning of immunodeficient mice to improve reconstitution

Nicole von Burg (2015)
Characterization of group 3 innate lymphoid cell function in the innate and adaptive immune system

Group Raphael Guzman
Xinzhou Zhu (2016)
The cold-inducible RNA-binding motif protein 3 (RBM3) prevents cell death through various mechanisms

Group Daniel Haag
Birte Boxler (2015)
On the epidemiology, biology and food-dependet reproduction of the feral pigeon (Columba livia)

Group Sinuhe Hahn
Maria Stoikou (2016)
Pharmacological modulation of neutrophil NETs

Chanchal Sur Chowdhury (2014)
Neutrophil extracellular traps in inflammatory disorders

Group Markus Heim
Vijay Shanker (2014)
Protein phosphatase 2A inhibits interferon signaling through the Jak STAT pathway and promotes hepatitis C viral replication

Group Christoph Hess
Marco Fischer (2015)
Mitochondria and effector functions of human CD8* T cells

Group Hans H. Hirsch
Piotr Kardas (2015)
JC and BK polyomavirus-like particles as targets of innate and adaptive humoral immunity

Group Georg Holländer
Simone Dertschnig (2014)
Breakdown of thymic tolerance - an etiologic link between acute and chronic graft-versus-host disease

Sanjay Gawade (2015)
Epigenetic regulation of thyroid development

Carlos Eduardo Mayer (2015)
Characterization of the spatio-temporal dynamics in thymic epithelial cell development

Group Giandomenica Iezzi
Francesca Amicarella (2014)
Immunobiology of IL-17A in human colorectal cancer

Eleonora Cremonesi (2016)
Chemotactic factors underlying tumor infiltration by immunocompetent cells in human colorectal cancer

Group Andreas Jehle
Jana Marina Orellana Miguez (2016)
Fatty Acids and their Metabolism in Diabetic Nephropathy and Podocytes

Group Josef Kapfhammer
Etsuko Shimobayashi (2016)
Mechanisms of PKC gamma-mediated inhibition of dendritic growth in cerebellar purkinje cells

Group Nina Khanna
Justyna Nowakowska (2014)
Different treatment approaches to infectious diseases: from novel antimicrobials to T-cell therapy

Group Thomas Klimkait
Joëlle Bader (2015)
Predictive power of HIV cell tropism on immunological recovery in HIV patients

Konstantin Kletenkov (2015)
The role of the HIV-1 protease substrate in therapy resistance

Group Stephan Krähenbühl
Andrea Felser (2014)
Mechanisms of hepatocellular toxicity associated with dronedarone and other mitochondrial toxicants

Group Matthias Liechti
Anna Rickli (2016)
Pharmacology of novel psychoactive substances

Group Raija Lindberg / Tobias Derfuss
Maria Zimmermann (2016)
B cells and endogenous retroviruses in multiple sclerosis

International PhD Program in Biomedicine

Dissertations 2014–2016

Group Luigi Mariani
Archana Ramadoss (2015)
Contribution of 3q26-29 gene cluster to glioma invasion

Group Ivan Martin
Carolina Maria Medeiros da Cunha (2014)
Mesenchymal stromal cell (MSC)-based control of angiogenesis and inflammation in cartilage formation

Atanas Atanasov Todorov (2015)
Endochondral ossification – towards a clinical translation

Group Albert Neutzner
Anne-Sophie Benischke (2014)
Ubiquitin-proteasome dependent mitochondrial protein quality control

Group Ed Palmer
Celine Gubser (2015)
Studies of suppression using monoclonal regulatory T cells and the importance of co-receptor Lck coupling ratios for negative selection

Lena Wyss (2015)
The role of TCR affinity for self-antigens in Helios positive versus Helios negative Tregs and their different distinct functional properties in maintaining self-tolerance

Group Olivier Pertz
Ludovico Fusco (2014)
Computer vision profiling of neurite outgrowth morphodynamics reveals spatio-temporal modularity of Rho GTPase signaling

Katrin Martin (2014)
Growth-factor-induced, persistent fibroblast migration is mediated by mechanical insulation of cell front and back

Group Daniel Pinschewer
Bénédict Fallet (2016)

Group Mike Recher
Laurent Schmied (2016)
Killer cell immunoglobulin-like receptors (KIR), licensing and ectosomes in the regulation of natural killer cell function: clinical implications and perspectives

Group Thérèse Resink
Agne Frismantiene-Petuskaite (2016)
Regulation of vascular smooth muscle cell function, behavior and signal transduction by T-cadherin

Kseniya Maslova (2014)
Modulatory effects of T-cadherin on cell behavior and growth factor receptor activity in carcinoma cells

Group Antonius Rolink
Lilly Audrey von Münchow (2016)
New insights into the molecular and cellular requirements of lymphocyte development

Group Michael Roth / Michael Tamm
Lei Fang (2015)
Neurodegenerative stress related mitochondrial proteostasis

Group Nicole Schaeren-Wiemers
Laetitia Sordé (2016)
Anti-inflammatory effects of intravenous immunoglobulin (IVIg): what are the mechanisms of action?

Group Primo Schär
Claudia Krawczyk (2014)
Novel functional aspects of topoisomerase Top1 and DNA Glycosylase Thp1 in the maintenance of genetic and epigenetic stability in Yeast

Melissa Manser (2016)
ELF-EMF perception and impact on epigenetic stability

Stefan Neu (2014)
Unravelling Molecular Mechanisms Underlying Genetic and Epigenetic Instabilities in Colorectal Cancer

Alain Weber (2015)
The biochemistry of DNA Oxidation- and repair-mediated active DNA demethylation

Stefan Weis (2015)
Unravelling molecular mechanisms underlying genetic and epigenetic instabilities in colorectal cancer

Annika Wirz (2014)
Linking active DNA demethylation by Thymine DNA Glycosylase with epigenetic regulation of gene expression

Group Jürg Schwaller
Katharina Leonards (2016)
The role of Nsd1 in hematopoiesis

Group Michael Sinnreich
Marielle Brockhoff (2016)
Deregulation of mTORC1 signalling and impairment of the autophagy process as pathomechanisms for Myotonic Dystrophy type I

Ruben Herrendorff (2015)
Novel therapeutic approaches for neuromuscular diseases

Group Giulio Spagnoli
Christian Kurt Hirt (2014)
Engineering the tumor microenvironment of colorectal cancer

Manuele Giuseppe Muraro (2014)
Functional validation of cancer stem cell markers in primary human colorectal cancer and established cell lines

Retreat 2016, Emmetten

Emanuele Trella (2015)
CD40L-expressing recombinant vaccinia virus (rVV40L): generation of central memory CD8+ T cells and apoptosis induction of tumor cells

Group Verdon Taylor
Robert Beattie (2014)

Anna Engler (2016)
Notch Signaling Mediates Neural Stem Cell Quiescene and Heterogeneity

Group Marten Trendelenburg
Arun Čumpelik (2014)
Microvesicles in diseases

Robert Kölm (2015)
Von Willebrand factor binds surface-bound C1q and induces platelet rolling.

Group Susan Treves / Thierry Girard
Rubén José López Dicuru (2016)
Study of calcium sparks in skeletal and smooth muscle cells in normal and pahtological conditions

Ori Rokach (2015)
The moleculare dysregulation of excitation contraction coupling in patients with congenital muscle disorders

Group Matthias Wymann
Fabrizio Botindari (2015)
Targeting SHIP1 and PI3Kγ for a synergistic inhibition of mast cell activation

Ruben Cal (2015)
Development of novel chemical inducers of dimerization to regulate proteins with high spatial and temporal precission

Anna Melone (2014)
Endosomal docking of mTOR modulates mTORC1 activity

Romy Walser (2014)
Non-canonical activation of PI3Kγ by Ca^{2+}/PKCβ in mast cells

Mirjam Sarah Zimmermann (2015)
Novel photocleavable intracellular heterodimerizer to manipulate protein dynamics with high spatiotemporal precision

Group Rolf Zeller
Sumit Jaiswal (2016)
Characterization of early mouse mesenchymal stem/progenitor cells for generating cartilage and study their role in endochondral ossification

Frédéric Laurent (2014)
Identification of cis-regulatory modules in mouse embryonic limb buds and heart using endogenous epitope-tagged transcription factors

Emanuele Pignatti (2014)
Targeting canonical BMP signaling: SMAD4 in limb patterning and differentiation

Erkan Uenal (2015)

Group Alfred Zippelius
Sandra Kallert (2016)
Interleukin-33 and Vaccine Vectors in Virus-Host Balance

Kea Simone Martin (2015)
Microtubule-depolymerizing agents potentiate anti-tumor immunity by stimulation of dendritic cells

Mission Statement

The mission of the Department of Biomedicine (DBM) at the University of Basel is to promote integrated research excellence in biomedical science.

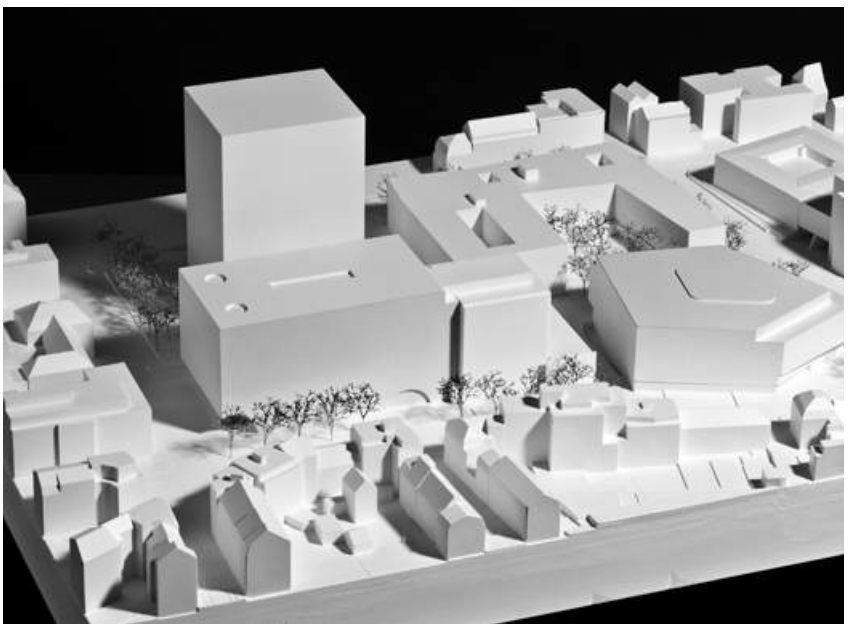
We emphasize research in neurobiology, infection and immunity, cancer, and stem cell biology and regenerative medicine.

The DBM provides a stimulating environment with state-of-the-art facilities, enabling biomedical research of the highest quality, while also training the next generation of leaders in basic and clinical research.

Central to our mission, non-clinical and clinical scientists work side by side to foster a translational environment across all of our disease-relevant scientific themes, benefiting the lives of patients in areas of unmet need.

New DBM Building

By 2024, all DBM researchers will work in a new building on the Schällemätteli Life Sciences Campus.



Modell: © Joseph Riegger

To further grow together as one department, all research groups, core facilities and staff of today's five locations will move under the same roof. The new DBM will complete the ongoing developments on the Schällemätteli Campus by joining the new buildings of the Biozentrum (2020) and the ETH Department of Biosystems Sciences and Engineering (2021) – creating a magnet for life science research in the Basel area with international reach. It will allow DBM researchers to interact more closely within the department but also with researchers of the neighbouring institutions. These interactions are further strengthened by jointly operated research core facilities, providing access to key technologies.

Visualisation: Caruso St John Architects

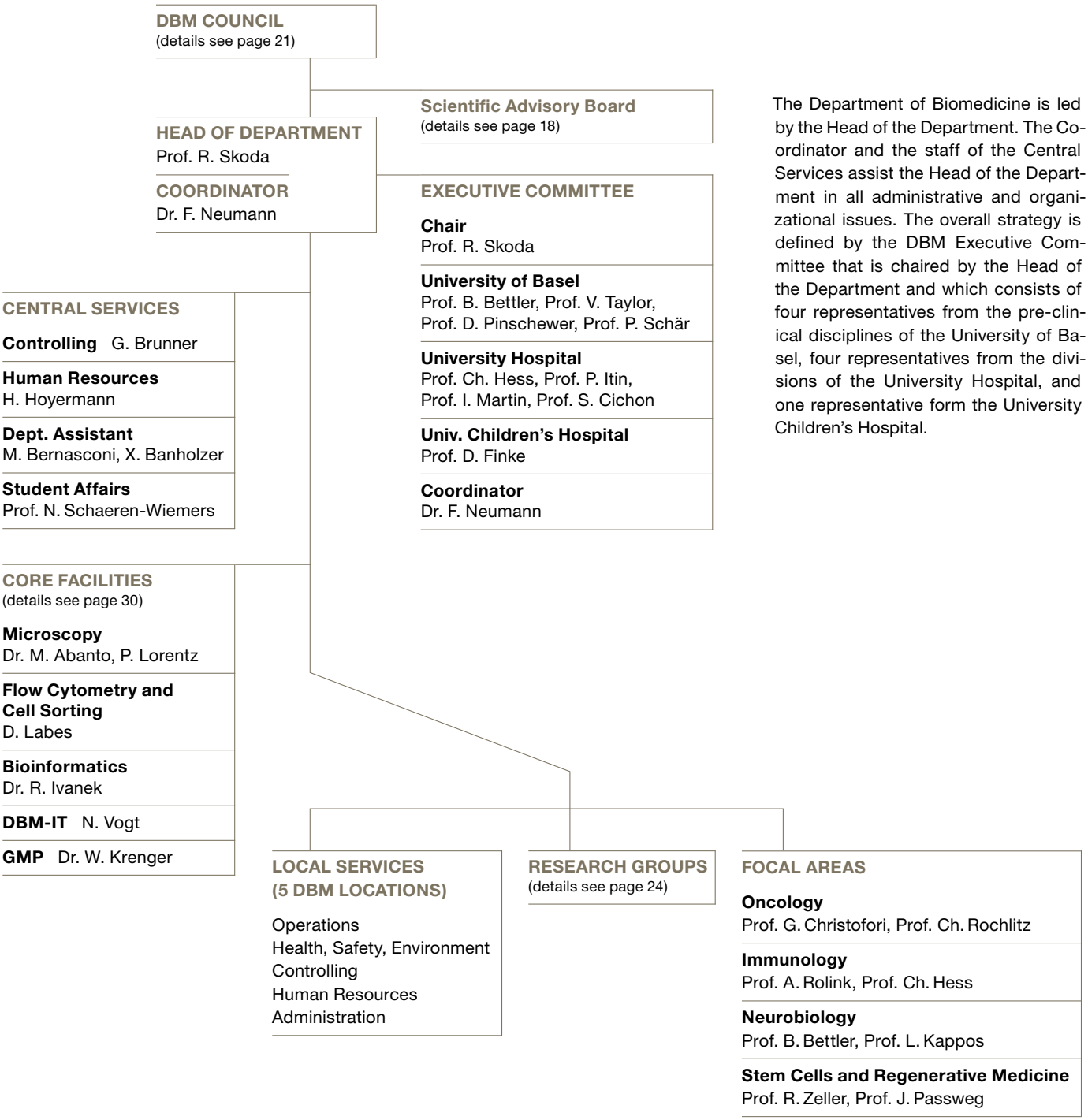
Foremost, the proximity of the DBM research labs to the University Clinics of the USB and UKBB will facilitate exchange and collaborations between scientists and physicians working on different disease areas, using complementary approaches and methodologies. This interdisciplinary exchange is the basis for a quick translation form bench to bedside in order for newly generated knowledge to be rapidly used in the development of new diagnostics or therapies for the benefit of patients.

The new DBM building will create synergies that lead to professionalization of technical and research services and a more efficient operation. These are the key for creating a competitive environment, where researchers can work more efficiently in state of the art laboratories, core facilities and infrastructures.

The project MCDLIX by Caruso St. John architects (Zürich and London) is the winning proposal of an anonymous competition for the new DBM building (2015). It convinced the jury with regard to urban planning, architectural character and the effective and flexible integration of the user requirements. The 7-floor building will have an effective area of 17'500 m2 and will be the new home for over 800 DBM employees. It will be owned and built by the University of Basel on a budget of 241 Mio CHF. The planning phase will last until 2019, realization until 2023 and the planned handover to the DBM is in 2023.

Further information can be found on the project website of the building department of the Canton of Basel Stadt: www.hochbauamt.bs.ch/projekte/laufende-projekte/dbm.html.

Organization



The Department of Biomedicine is led by the Head of the Department. The Coordinator and the staff of the Central Services assist the Head of the Department in all administrative and organizational issues. The overall strategy is defined by the DBM Executive Committee that is chaired by the Head of the Department and which consists of four representatives from the pre-clinical disciplines of the University of Basel, four representatives from the divisions of the University Hospital, and one representative form the University Children's Hospital.

Key Data 2016

RESEARCH GROUPS	**67**
CORE FACILITIES	**11**
DBM Core Facilities	4
Joint Core Facilites	7
SPACE	
Locations	5
Effective area	13’608 m^2
PERSONNEL	
Employees total	837
FTE (total)	648
FTE (third-party funded)	344
Research group leaders	77
Tenured Professors	41
SNSF Professors	7
Titular- and Tenure track-assistant professors	15
PD and SNSF Ambizione SCORE	14
PhD Students	122
FINANCES (in CHF)	
Personnel	29’801’526
Supplies	9’865’792
Investments (equipment)	4’711’789
Income (total)	-8’694’734
Third-party funds (grants etc.)	22’504’113

Open-space laboratory at DBM-Hebelstrasse.

Scientific Advisory Board

The Scientific Advisory Board comprises academics of international reputation who do not belong to the University of Basel. At least two members are designated for the Advisory Board for each focal area. Clinical and non-clinical academics must be suitably represented on the Advisory Board.

In addition to the permanent members of the Advisory Board, subject-specific experts can be called in to appraise the focal areas.
The Scientific Advisory Board advises the DBM Executive Committee, particularly on issues of research quality and organization.

The Scientific Advisory Board meets once a year. It compiles a report for the attention of the DBM Council that is also provided to the DBM Executive Committee, the Dean's Office, the Rectorate, and to the directors of the university hospitals involved in the DBM. It is also responsible for its own organization. The DBM Executive Committee provides the Advisory Board with administrative support. The Advisory Board may also be consulted by the DBM Council during the year in the event of upcoming decisions.

Immunology and Infection

Prof. Kathryn Wood
chair
Nuffield Department of Surgery,
John Radcliffe Hospital, Headington,
Oxford, UK

Prof. Brigitta Stockinger
Division of Molecular Immunology,
MRC National Institute for Medical
Research, London, UK

Neurobiology

Prof. Christian Lüscher

Département des Neuro-sciences Fondamentales & Service de Neurologie, Centre Medical Universitaire Genève, Switzerland

Prof. Christian Rosenmund

Charité Neurowissenschaftliches Forschungszentrum, Max Delbrück Center for Molecular Medicine, Berlin, Germany

Stem Cells and Regenerative Medicine

Prof. Giulio Cossu

Constance Thornley Professor of Regenerative Medicine, The University of Manchester, UK

Prof. Karl-Heinz Krause

Departments of Pathology, Immunology and Clinical Pathology, Faculty of Medicine & University Hospitals of Geneva, Geneva, Switzerland

Oncology and Cancer Research

Prof. Owen Sansom

Cancer Research UK, Beatson Institute and University of Glasgow, Glasgow, UK

Prof. Ivo Touw

Department of Hematology, Erasmus University Medical Center, Rotterdam, The Netherlands

Left during report period:
Greg Lemke, Margaret Frame, Paolo Bianco

Joining in 2018:
Pamela Ohashi, Bernhard Malissen, Klaus-Armin Nave

Executive Committee

Prof. Radek Skoda
Head of the Department

Prof. Bernhard Bettler

Prof. Sven Cichon

Prof. Daniela Finke

Prof. Christoph Hess

Prof. Peter Itin

Prof. Ivan Martin

Prof. Daniel Pinschewer

Prof. Primo Schär

Prof. Verdon Taylor

Dr. Frank Neumann

Council of the Department

(DBM Rat)

From left to right

Prof. Edwin C. Constable (Vice President, University of Basel)

Dr. Werner Kübler (Director, University Hospital Basel)

Prof. Radek Skoda (Head, Department of Biomedicine – without voting right)

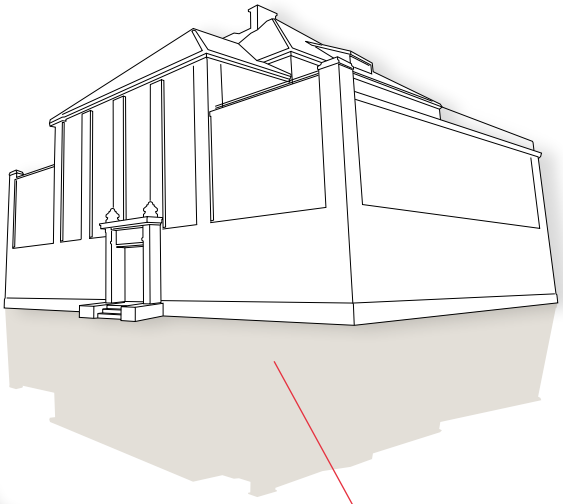
Christoph Tschumi (Head of Administration, University of Basel)

Prof. Thomas Gasser (Dean of the Faculty of Medicine, University of Basel)

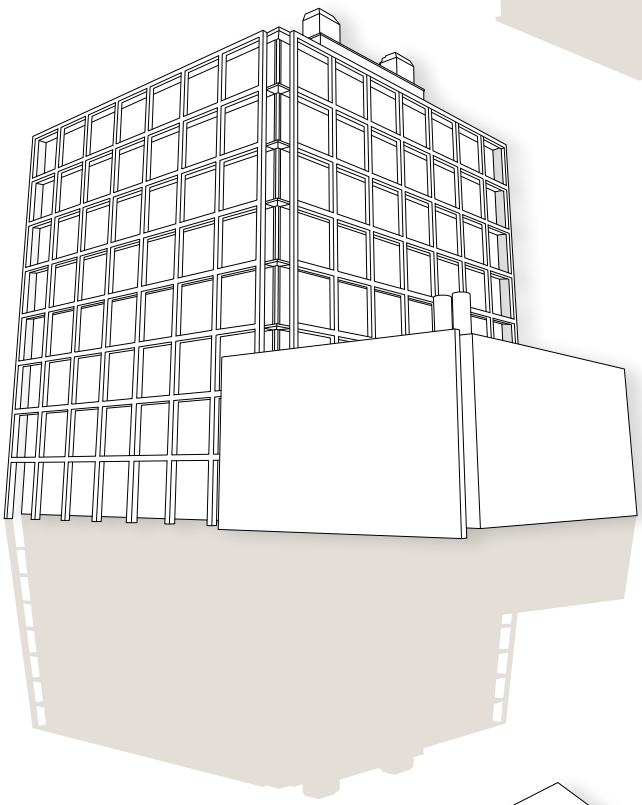
Marco Fischer (Director, University Children's Hospital Basel)

Dr. Kaspar Traub (Head of Administration of the Faculty of Medicine, University of Basel) (not on this picture)

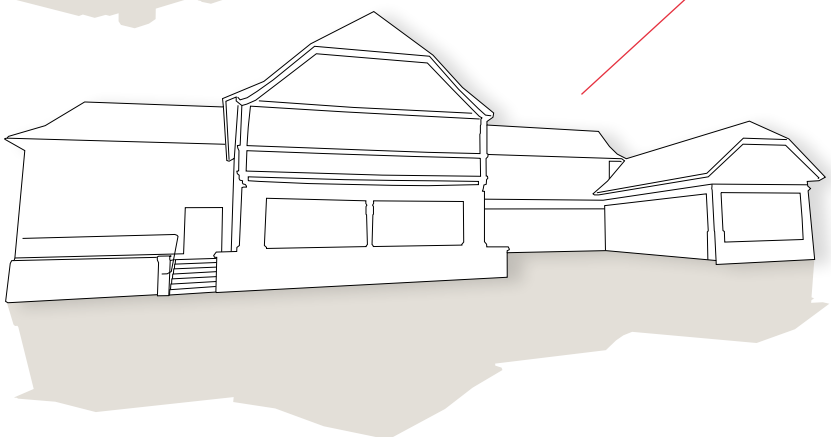
Locations



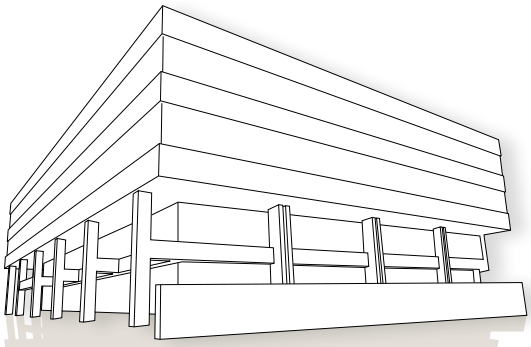
Pestalozzistrasse 20
4056 Basel



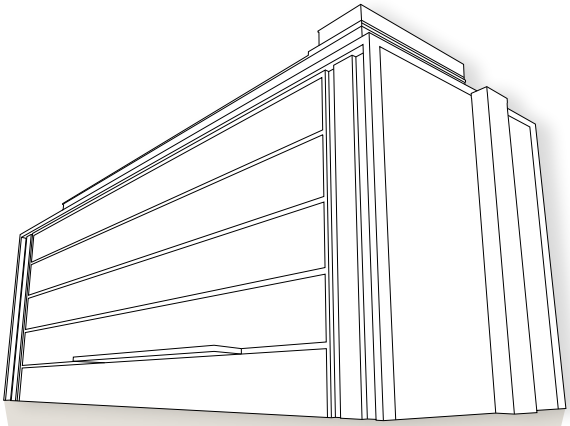
Klingelbergstrasse 50/70
Pharmazentrum (7th floor)
4056 Basel



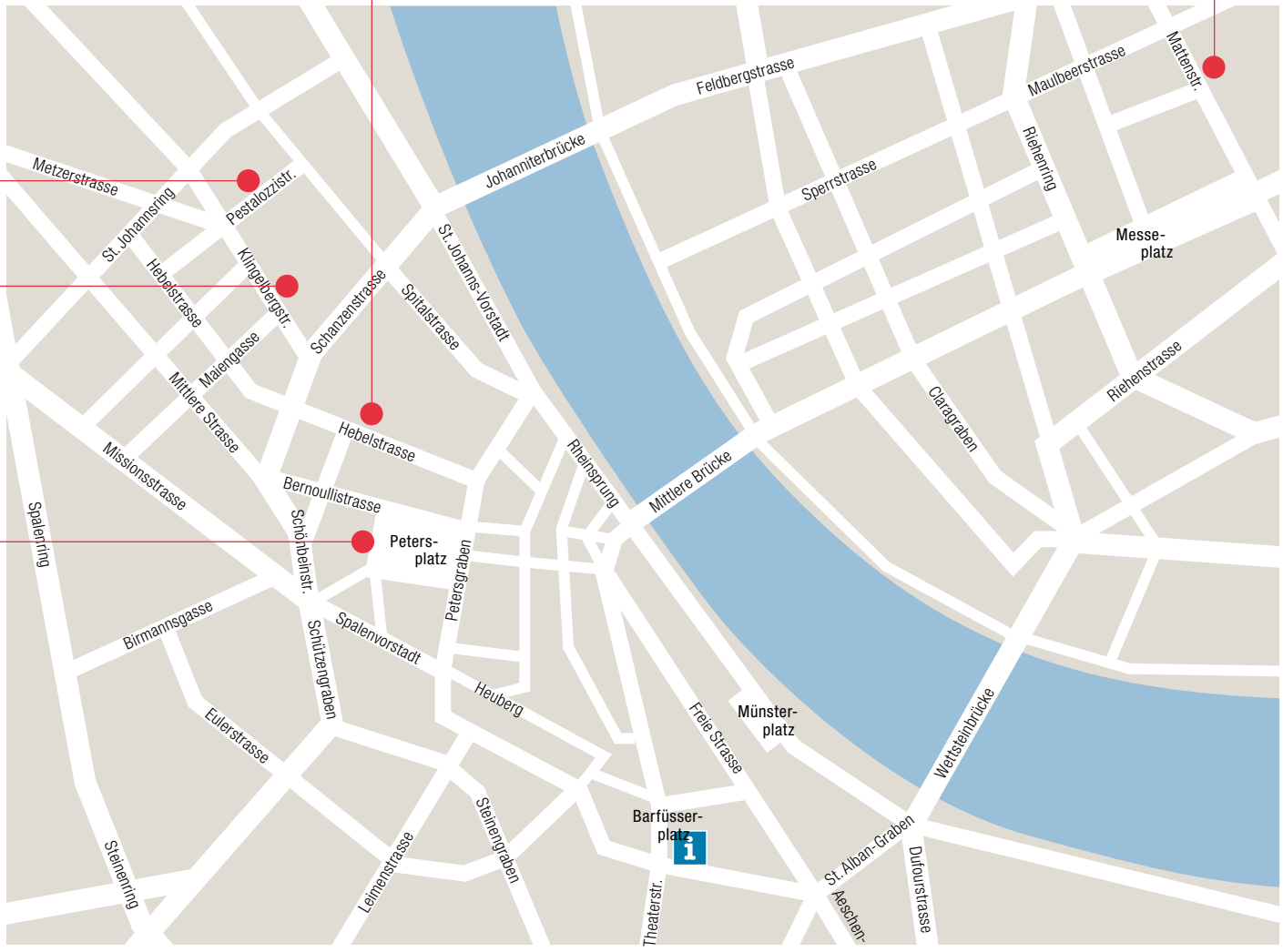
Petersplatz 10
4001 Basel



Hebelstrasse 20
4031 Basel



Mattenstrasse 28
4058 Basel



Research Groups

Overview according to location
and focal area

Department of Biomedicine
Hebelstrasse 20

PD Dr. Andreas Banfi
Cell and Gene Therapy

Prof. Mohamed Bentires-Alj
Tumor Heterogeneity,
Metastasis and Resistance

PD Dr. Christoph Berger
SNF Ambizione SCORE
Translational Immunology

Prof. Daniel Bodmer
Inner Ear Research

Prof. Marijke Brink
Prof. Peter Buser
Cardiobiology

PD Dr. Claudia Cavelti
SNF Ambizione SCORE
Translational Diabetes

Prof. Sven Cichon
Human Genomics

Prof. Christian DeGeyter
Gynecological Endocrinology

Prof. Gennaro De Libero
Experimental Immunology

Prof. Tobias Derfuss
Prof. Raija Lindberg
Clinical Neuroimmunology

Prof. Marc Donath
Diabetes Research

PD Dr. Adrian Egli
SNF Ambizione SCORE
Applied Microbiology Research

PD Dr. Magdalena Filipowicz Sinnreich
Liver Immunology

Prof. Raphael Guzman
Brain Ischemia and Regeneration

Prof. Sinuhe Hahn
Prenatal Medicine

Prof. Markus H. Heim
Hepatology

Prof. Viola Heinzelmann
Ovarian Cancer Research

Prof. Christoph Hess
Immunobiology

Prof. Giandomenica Iezzi
SNF Professorship
Cancer Immunotherapy

Prof. Peter Itin
Dermatology

Prof. Lukas Jeker
SNF Professorship
Molecular Immune Regulation

Prof. Beat Kaufmann
Cardiovascular Molecular
Imaging

PD Dr. Nina Khanna
SNF Ambizione SCORE
Infection Biology

Prof. Carolyn King
SNF Professorship
Immune Cell Biology

Prof. Stephan Krähenbühl
Clinical Pharmacology

PD Dr. Gabriela Kuster Pfister
Prof. Stefan Osswald
Myocardial Research

Prof. Claudia Lengerke
Stem Cells and Hematopoiesis

PD Dr. Matthias Liechti
Psychopharmacology Research

PD Dr. Anna Marsano
Prof. Friedrich Eckstein
Cardiac Surgery and Engineering

Prof. Ivan Martin
Tissue Engineering

PD Dr. Matthias Mehling
SNF Ambizione SCORE
Translational Neuroimmunology

Dr. Sara Meyer
SNF Ambizione SCORE
Myeloid Malignancies

PD Dr. Albert Neutzner
Prof. Hendrik Scholl
Ocular Pharmacology
and Physiology

Prof. Jan Hendrik Niess
Gastroenterology

Prof. Mike Recher
SNF Professorship
Immunodeficiency

Prof. Therese J. Resink
Prof. Paul Erne
Signal Transduction

Prof. Michael Roth
Prof. Michael Tamm
Pneumology

Prof. Nicole Schaeren-Wiemers
Neurobiology

Prof. Jürg Schwaller
Childhood Leukemia

Prof. Radek Skoda
Experimental Hematology

Prof. Giulio C. Spagnoli
Oncology Surgery

Prof. Marten Trendelenburg
Clinical Immunology

Prof. Susan Treves
Prof. Thierry Girard
Perioperative Patient Safety

Prof. Alfred Zippelius
Prof. Christoph Rochlitz
Cancer Immunology and Biology

Department of Biomedicine
Mattenstrasse 28

Prof. Nicola Aceto
SNF Professorship
Cancer Metastasis

Prof. Gerhard Christofori
Tumor Biology

Prof. Daniela Finke
Developmental Immunology

Prof. Georg A. Holländer
Pediatric Immunology

Dr. Javier Lopez-Rios
Development and Evolution

Prof. Antonius Rolink
Developmental and Molecular Immunology

Prof. Primo Schär
Genome Plasticity

Prof. Verdon Taylor
Embryology and Stem Cell Biology

Prof. Roxane Tussiwand
SNF Professorship
Immune Regulation

Prof. Matthias Wymann
Cancer- and Immunobiology

Prof. Rolf Zeller
PD Dr. Aimée Zuniga
Developmental Genetics

Department of Biomedicine
Pestalozzistrasse 20

Prof. Josef Bischofberger
Cellular Neurophysiology

Prof. Daniel Haag-Wackernagel
Integrative Biology

Prof. Josef Kapfhammer
Developmental Neurobiology and Regeneration

Prof. Magdalena Müller-Gerbl
Musculoskeletal Research

Prof. Eline Pecho-Vrieseling
SNF Professorship
Neuronal Development and Degeneration

Department of Biomedicine
Klingelbergstrasse 50/70

Prof. Bernhard Bettler
Molecular Neurobiology
Synaptic Plasticity

Prof. Luigi Mariani
Brain Tumor Biology

Prof. Tania Barkat Rinaldi
Brain and Sound

Prof. Michael Sinnreich
Neuromuscular Research

Department of Biomedicine
Petersplatz 10

Prof. Hans H. Hirsch
Transplantation and Clinical Virology

Prof. Thomas Klimkait
Molecular Virology

Prof. Diego Kyburz
Rheumatology

Prof. Daniel Pinschewer
Experimental Virology

Core Facilities

Microscopy
Dr. Mike Abanto
Pascal Lorentz

Flow Cytometry and Cell Sorting
Danny Labes

Bioinformatics
Dr. Robert Ivanek

GMP Facility
Dr. Werner Krenger

Anatomy Museum
Prof. Magdalena Müller-Gerbl

Diagnostic Services

PCR / HIV Laboratory
Prof. Hans H. Hirsch

Serology / Virology Labaratory
Prof. Thomas Klimkait

HLA Testing Laboratory
Prof. Jürg Steiger

Medical Genetics Laboratory
Prof. Sven Cichon

Pharmacology and Toxicology
Prof. Stephan Krähenbühl

Legend

- Neurobiology
- Stem Cells and Regenerative Medicine
- Oncology and Cancer Research
- Immunology and Infection

Newly Apppointed Professors 2014–2016 Faculty

Mohamed Bentires-Alj

born in 1972 (Casablanca, Morocco), studied Pharmaceutical Sciences at the University of Liège, Belgium (1991–96), and obtained a PhD in Pharmaceutical Sciences at the same university in 2001. After a postdoc at BIDMC, Harvard Medical School, Boston, USA (2001–06), he was a group leader at the Friedrich Miescher Institute in Basel (2006–16) and an ERC fellow (staring grant). In September 2016, he was appointed Professor of Experimental Surgical Oncology at the Department of Biomedicine, University of Basel. He is an ERC fellow (advanced grant). His research is focused on mechanisms regulating normal and neoplastic breast stem cells, metastasis, and resistance and on personalized medicine.

Jan Niess

Jan Hendrik Niess, born in 1971 (Erlenbach, Germany), studied Medicine at the Friedrich Schiller University Jena, Germany (1993-2000). He worked then as a postdoc at the Gastrointestinal Unit, Center for the Study of Inflammatory Bowel Disease (CSIBD), Massachusetts General Hospital and Harvard Medical School. He was the trained in Internal Medicine and Gastroenterology at the University Hospital Ulm before he moved to Bern, where he worked as a Consultant in Gastroenterology and as a research group leader at the Inselspital, Bern. He is a Tenure Track Assistant Professor for Gastroenterology in Basel. His research is focused on pathways how the host recognizes parts of the microbiota.

Newly Apppointed Professors 2014–2016 Junior Faculty

Nicola Aceto

Nicola Aceto, born 1982 in Alessandria, Italy, obtained his PhD from the Friedrich Miescher Institute in 2011, with a thesis on the role of protein tyrosine phosphatases in breast cancer. He then moved to Harvard Medical School and Massachusetts General Hospital Cancer Center (Boston, MA, USA) where he worked on circulating tumor cells and cancer metastasis. He was then awarded an ERC-Starting grant and a Professorship from the Swiss National Science Foundation to start his own group in Basel. His research combines microfluidic technologies, patient samples, mouse cancer models, molecular and computational biology to study circulating tumor cells and to identify vulnerabilities of the metastatic process.

Claudia Cavelti-Weder

Claudia Cavelti-Weder, born 1978 in Bern, Switzerland, graduated from Medical School in Basel in 2004 and completed her Internal Medical Residency and later Endocrinology Residency at the University Hospital of Zurich. She spent her postdoctoral fellowship in Boston at the Joslin Diabetes Center of Harvard University (2010–2013) and acquired a Master in Public Health at Harvard University. Since 2014, she is working as a senior physician in Endocrinology at the University Hospital of Basel. She was awarded the Ambizione grant in 2015 and started her own research group with a focus on the role of immune cell activation in metabolic disease.

Adrian Egli

Adrian Egli, born 1978 in Basel, Switzerland, graduated as MD from Medical School in Basel in 2004. He obtained a PhD degree in 2008. In 2005, and 2009-2010 he worked in Internal Medicine, Haematology and Infectious Diseases at the University Hospital Basel. After a postdoctoral fellowship (2011–2012) at the University of Alberta, in Edmonton, Canada, Adrian finished his training in Clinical Microbiology (2013-2015). He was awarded with the SNSF Ambizione grant and SystemsX grant in 2014 and started his own research group “Applied Microbiology Research”. Adrian’s research focuses on immune responses and pathogen transmission. He was habilitated in 2015. Since 2015, he is the Head of Division of Clinical Microbiology at the University Hospital Basel.

Magdalena Filipowicz Sinnreich

Magdalena Filipowicz Sinnreich, born 1979 in Warsaw, Poland, graduated as MD from Medical School in Basel in 2004. She obtained a PhD degree within the MD-PhD program in 2008, and completed clinical training in Internal Medicine and Gastroenterology/Hepatology in Basel and Liestal. She worked as postdoctoral fellow in the Immunobiology Laboratory at the University Hospital Freiburg, Germany, and received the Venia Docendi at the University of Basel in 2014. While working as Gastroenterology/Hepatology specialist at the University Medical Clinic in Liestal, she was awarded the Goldschmidt-Jacobson Foundation and SNSF Ambizione-Score grants in 2016 to study the role of Mucosal-associated invariant T (MAIT) cells in liver diseases.

Newly Apppointed Professors 2014–2016 Junior Faculty

Carolyn King

Carolyn King, born in 1975 in New Jersey, USA, studied biology at Duke University. After receiving her degree in 1997 she moved to San Francisco and worked for a year as a business analyst for McKesson followed by a technician position at the Gladstone Institute and in Kampala Uganda. In 2001 she began graduate work at University of Pennsylvania working on the role of E3 ubiquitin ligases in tolerance and immunity. In 2007 she began a postdoctoral fellowship at the Department of Biomedicine in Basel. In 2013 she received an Ambizione fellowship which she spent at both the Department of Biomedicine and later at the D-BSSE ETH Zurich. In 2016 Carolyn began a professorship funded by the Swiss National Science Foundation. Her primary research interest is in understanding how individual white blood cells of the immune system give rise to diverse and specialized fates.

Javier Lopez-Rios

Javier Lopez-Rios, born 1974 in Madrid, Spain, graduated in Biology at the Autónoma University of Madrid. As a postgraduate, he studied the early specification of retinal progenitors. After earning his PhD in 2002, he did a postdoctoral stage at the Cajal Institute. In 2005, he joined the group of Rolf Zeller at the DBM as a postdoctoral fellow to study the genetic networks operating in limb development. In 2015, he was appointed Group Leader at the Department of Biomedicine. His research combines genetic analysis in the mouse with functional genomics in several vertebrate species to study the cis-regulatory control of limb morphogenesis in the contexts of evolution and human congenital defects.

Anna Marsano

Anna Marsano, born in 1976 in Genova, Italy, studied Biomedical Engineering at the University of Genova. In 2006 she obtained her PhD from the EPFL, CH. From 2006 until 2009 she was a postdoctoral fellow at the Columbia University in NY, US, where she trained in cardiac tissue engineering and regeneration. She then worked at the DBM in Basel first in Martin's group and since 2012 as project leader in Banfi's group working on different strategies to control *in vivo* angiogenesis. In 2014 she was recognized as Research Group Leader at the DBM. Her work focuses on the development of cell-based angiogenic therapies to treat chronic cardiac ischemia and of 3D cardiac models to investigate basic cellular mechanisms.

Matthias Mehling

Matthias Mehling, born 1976 in Eichstätt, Germany, graduated from University of Tübingen Medical School in 2004 and trained at the Neurology Department of the University Hospital Basel from 2005 until 2011. Following this, he worked as a postdoctoral fellow at the Department of Biosystems Science and Engineering of ETH Zurich (2012-2014) and the Institute of Science and Technology Austria (2015). He was awarded a Swiss National Science Foundation Ambizione-Score grant in 2015 and was habilitated in 2016. His research focuses on assessing migration characteristics of human T cells sampled from healthy subjects and patients with multiple sclerosis on the single cell level.

Sara Meyer

Sara Meyer, born 1979 in Baden, Switzerland, graduated from Medical School at University of Bern 2005 (MD 2009). After training in experimental hematology at University of Bern and Children's Hospital of Philadelphia, she obtained her PhD 2009. She did her clinical training in internal medicine and hematology in Baden and Basel. During her postdoctoral fellowship at Memorial Sloan Kettering Cancer Center New York from 2012–2015, she focused on JAK2 signaling in myeloproliferative neoplasms and resistance to JAK2 inhibitors. Upon her return to University Hospital Basel in 2015, Sara Meyer was awarded an SNF Ambizione-SCORE Grant for her studies on improved targeting of oncogenic signaling in myeloid malignancies.

Eline Pecho-Vrieseling

Eline Pecho-Vrieseling, born 1977 in Wijns, the Netherlands, studied Biology with Biomolecular Sciences at the University of Amsterdam, the Netherlands. She graduated 2006 from the University of Basel with a doctorate in Neuroscience. She continued another 2 year as a postdoc at the University of Basel, after which she received a presidential postdoc fellowship at Novartis Institute for Biomedical Research, Basel. In 2016 she received a Swiss National Science Foundation professorship and will join the Department of Biomedicine in November 2016. Her research interest is to understand the role and mechanism of cell-to-cell spreading of pathogenic proteins in neurodegenerative disorders.

Tania Rinaldi Barkat

Tania Rinaldi Barkat, born 1977 in Wallis, Switzerland, graduated in Physical Chemistry from the EPFL in 2001. She then switched to the field of neuroscience and completed a PhD in the same institution in 2006. She spent her postdoctoral fellowship at Harvard, Boston, where she studied a critical period for plasticity in the auditory system. In 2013 she started her independent path at Copenhagen University, and joined Basel University as a tenure-track professor in 2015. In addition to being elected member of the Harvard Society of Fellows, Tania was awarded a Lundbeck Foundation Fellowship and an ERC Starting grant. Her work focuses on the development and function of neuronal circuits in the auditory system.

Roxane Tussiwand

Roxane Tussiwand, born in 1976 in Mashhad, Iran, studied biology at the University of Milan. In 2000 she worked as research associate for the Research institute Fondazione Tettamanti and graduated in 2002 working on the prenatal origin of childhood leukemia. In 2006 she obtained an international PhD in molecular medicine sponsored by the Italian government at the Institute for Research in Biomedicine in Bellinzona, Switzerland, working on dendritic cell development. In 2006 she joined the laboratory of A. Rolink at the Department of Biomedicine (DBM) in Basel as a postdoctoral fellow studying early hematopoietic development. In 2010 she moved to Washington University in St. Louis, USA, where under the supervision of K. Murphy she worked on the transcriptional regulation of dendritic cell development. In October 2004 she started her research group funded by the Swiss National Science Foundation at the DBM in Basel. Her main research interest is in understanding the development and function of dendritic cells.

Core Facilities of the Department of Biomedicine

Scientific core facilities are an integral part of the DBM research landscape. They enable cutting-edge research by helping to economically and efficiently take advantage of state-of-the-art technology and collaborate with expert staff.

The highly trained facility members serve the evolving needs of the DBM research groups by following up with the latest technological advances, giving comprehensive trainings to the users or by coordinating grant applications to purchase new equipment. DBM core facilities are providing their service at no or very little costs, minimizing the administrative burden and leveraging resources to support investigator-driven science. Dedicated steering committees evaluate the services and provide guidance to the core facility heads in order to improve the core facility infrastructure, technology and management for the benefit of the DBM research groups.

While DBM members have access to in-house core facilities for bioinformatics, flow cytometry & cell sorting, light microscopy and for the production of medicinal products according to Good Manufacturing Practices (GMP), the DBM joins forces with other life sciences institutions in Basel to run core facilities for next generation sequencing technologies and genomics research, design and generation of custom transgenic rodent models, preclinical nuclear molecular imaging, electron microscopy, and high-performance computing.

The DBM has a long history in sharing and centralizing common equipment to leverage resources. In the absence of dedicated core facility personnel, these facilities are maintained by members of DBM research groups or by technical staff. Such technologies that are available to all researchers include facilities for histology, robotics, tissue culture, biobanking and other high-end applications.

Together, these core facilities play a central role in creating a competitive research environment and contribute significantly to the scientific success of the Department of Biomedicine.

Storage of biological samples in liquid nitrogen.

Good Manufacturing Practice (GMP)

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Introduction
Good Manufacturing Practice (GMP) is a system to ensure that medicinal products intended for human use are consistently produced and controlled according to appropriate quality standards. Swiss manufacturers of medicinal products must comply with international GMP standards set forth by the European Union (EU). These GMP standards are built on three pillars: 1) Pharmaceutical substances and products intended for human use are to be manufactured in sites that are adequately equipped, 2) the producer has to demonstrate appropriate professional and technical knowledge that is provided by qualified staff, and 3) a pharmaceutical Quality Assurance system needs to be established by the manufacturer.

Service
The DBM has obtained an permit from Swissmedic in March 2015 to operate such a manufacturing laboratory. Headed by Werner Krenger, PhD, the "GMP core facility" is located on the 4th floor of the ZLF and offers as "Provider" central services to individual DBM research groups ("Users) that permit the manufacture of investigational medicinal products (IMPs) to be used in clinical trials. A delimitation agreement between Provider and User regulates activities and responsibilities. The final product is released for clinical use by the the head of the core facility.

Affiliation and Staff
Both the core facility provider and the DBM research group users are required to contribute to the implementation of a fully functional Quality Assurance (QA)-system necessary for the release of GMP-compliant medicinal products: A QA-team is provided by the GMP core facility DBM and includes the "Qualified Person" (W. Krenger) with his deputy PD Dr. Paul Zajac, the "QA manager" Anke Wixmerten; and an external quality assurance consulting firm "Quality Assurance Consulting AG; QAC). The second team needs to be provided by the DBM research groups and includes at minimum a "Quality Control Manager" (QC), a "Manufacturing Manager" (MM) and a "Product Manager" (PM; which is usually the principal investigator of a research group). Together, the teams establish the relevant documentation required for implementation of an appropriate production process. Training of all staff (both core facility staff and research group staff) is organized by the core facility staff.

Equipment
The laboratory, including its supporting utilities (computerized GMP monitoring system) and the equipment (incubators, fridges, freezers, centrifuges, air sampler) have been qualified in accordance with the requirements of GMP.

Outlook
Since its inception in March 2015, two DBM research groups have used the core facility services to produce different IMPs (cartilage tissue and genetically engineered vaccinia virus; Fig. 2) to be used in two clinical trials. The facility offers, however, the capacity for including additional teams. The DBM hopes that the availability of this core facility will further stimulate the transition of preclinical research into the study of exciting new investigational medicinal products.

Production of genetically engineered vaccinia virus for clinical use.

Bioinformatics

The Bioinformatics Core Facility provides a centralized resource of expertise in computational biology and statistics, available to all researchers at the DBM. It offers support for the analysis and visualization of large-scale biological data, mainly produced by high-throughput genomics experiments. The platform also provides training in bioinformatics and facilitates access to high-performance computational resources.

Design and analysis of the high-throughput biological data sets

The aim of the Bioinformatics Core Facility is to implement solutions for analysis, interactive visualization, management and interpretation of large-scale genomic data generated by high-throughput techniques derived from human or model organisms. Over the past four years, the Bioinformatics Core Facility analyzed data of more than 100 studies from 43 research groups on gene expression, DNA-protein binding (ChIP-, CLIP-seq), DNA methylation, DNA accessibility, mapping of physical contacts between genomic elements (4C-seq), identification of sequence variants or high-throughput screens. Beside supporting standardized approaches, the platform also develops customized solutions tailored towards the needs of individual research projects.

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Bioinformatics training

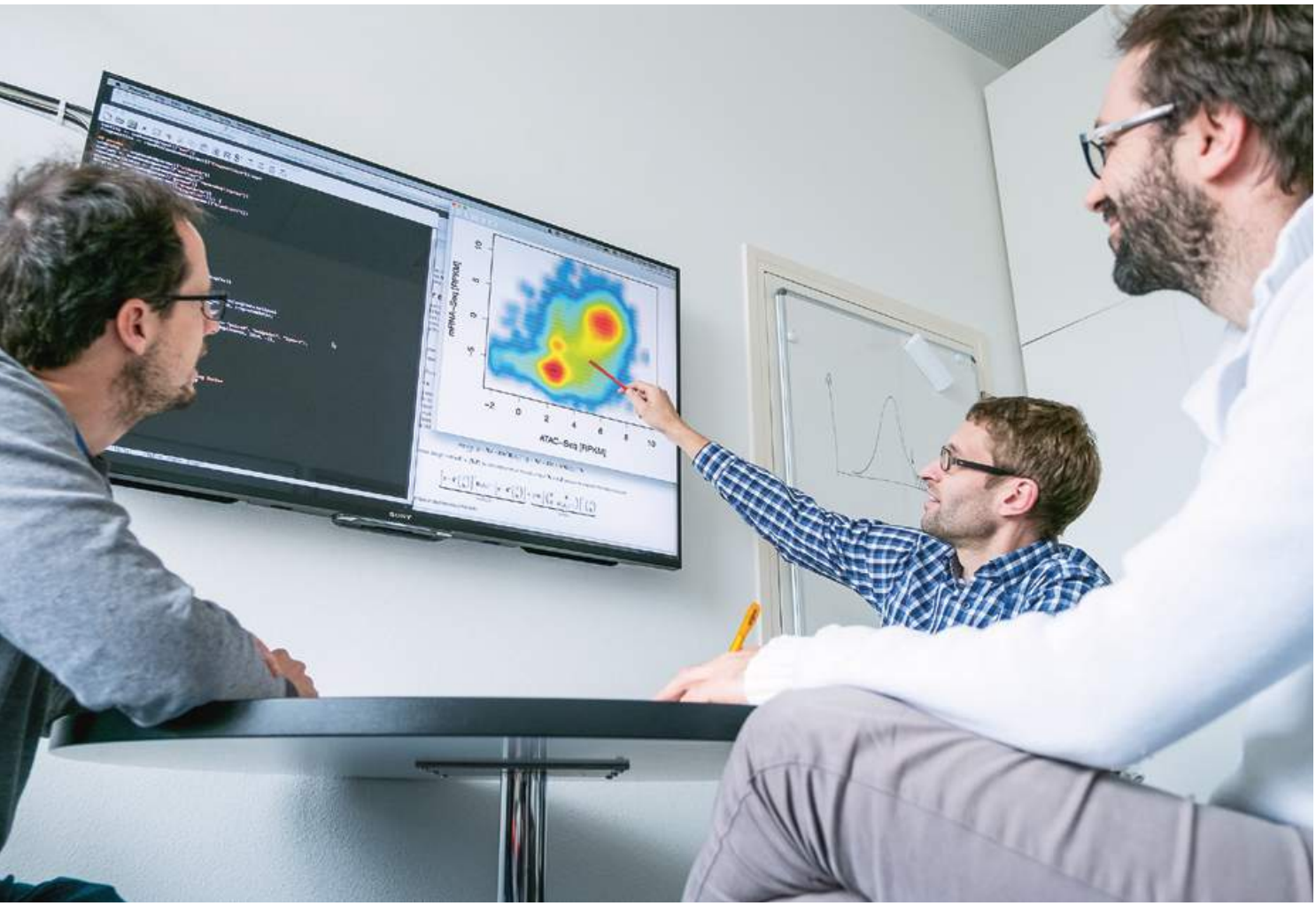
The Bioinformatics Core Facility is individually training and advising researchers at the DBM. Furthermore, Robert Ivanek as group leader of the Swiss Institute of Bioinformatics (SIB) is regularly mentoring the bioinformaticians that are embedded within the DBM research groups.

The facility also organizes bioinformatics trainings in collaboration with computational biologists from the Friedrich Miescher Institute for Biomedical Research. These trainings comprise two full semester lecture series and exercises:

1. "Introduction to **R**", a hands-on course on the basic usage of **R** software with a focus on data exploration and visualization, and basic statistics.
2. Advanced course "Analysis of genomics data in **R** and Bioconductor" gives students an overview of typical Bioconductor based analysis pipelines including: NGS data processing, differential gene expression analysis, DNA binding analysis, working with sequence and annotation packages and visualization of genomic data.

Infrastructure

The platform's mission is to build and maintain an infrastructure that enables application of strong bioinformatics analysis to enhance and empower the biomedical research at the DBM. To keep track of the rapid technological advances and the growing number of bioinformatics approaches and tools, the Bioinformatics Core Facility closely interacts with other bioinformatics units in the Basel area (e. g. the group of Dr. Michael Stadler, Friedrich Miescher Institute for Biomedical Research and the group of Prof. Torsten Schwede and the center for scientific computing – sciCORE at the University of Basel).



Project discussion at the Bioinformatics Core Facility.

Microscopy

The Microscopy Core Facility is a centralized platform that provides cutting-edge microscopes and expert support to all DBM researchers.

The field of light microscopy is dynamic and rapidly evolving. Just in in 2014 Eric Betzig, Stefan W. Hell and William E. Moerner shared a Nobel Prize for 'super-resolution' microscopy. Before this invention, microscopes were limited to imaging at the micrometer-scale; super-resolution microscopy now pushes this limit to the nanometer range – creating new the possibilities for scientific discoveries. This field is just one example where the microscopy core facility has greatly facilitated access to a new technology for DBM researchers by providing the expertise required to purchase, operate and maintain a super-resolution microscope.

Infrastructure and equipment

The facility staff is currently supervising over 20 high-end microscopes, including super-resolution, confocal, widefield, FRAP, TIRF, high content screening, slide scanning, live-cell imaging, flow cytometer imaging, stereo, and laser capture microdissection. The facility also provides resources for image analysis, including high-end computers, virtual machines, data and image analysis servers, and several commercial and open-source image analysis softwares.

Training and support

The facility trains and advises researchers throughout the entire microscopy process, from initial idea to publication. Support often starts with a conversation about the biology and leads to a robust microscopy experimental design that includes sample preparation, choice of microscope, software for image visualization, and strategy for analysis. Researchers are then trained to work independently at the corresponding microscopes and computers.

Future developments

In order to learn and develop new microscopy technology, the facility is embedded in European and Swiss microscopy networks. In the Basel area, the academic microscopy facilities are organized within the Microscopy Network Basel (MNB, https://microscopynetwork.unibas.ch/home/). The MNB facilitates interactions on the levels of sharing resources and knowledge, fostering collaborations and co-organizing courses, equipment demonstrations, seminars or symposia.
While the core facility staff is constantly keeping up with the latest technological developments, a steering committee of DBM research group leaders is providing instrumental guidance. This allows the facility swiftly adapt to the changing and developing needs of the DBM research community.

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Live cell imaging using microfluidics technology.

Flow Cytometry

Flow Cytometry is a key technology for many DBM research groups in the focal areas immunology & infectious disease, oncology & cancer research and stem cells & regenerative medicine. The DBM Flow Cytometry Facility (FCF) provides high-end infrastructure and expertise for cell sorting and flow cytometric analyses.

To analyze prokaryotic or eukaryotic cells, organelles or chromosomes, the suspended particles are injected into a constantly flowing sheath stream that passes along several laser beams and a sensitive fluorescence detection area. Each cell that is passing this area can be examined for the presence of multiple fluorescent probes at rates of several ten thousand events per second. Real-time data analysis provides exact statistics about signal intensity and frequency of each cell and multiple subpopulations. These can be analyzed directly or further isolated by Fluorescence Activated Cell Sorting (FACS).

Training and service

The facility is run by specialized and highly trained FACS operators who assure that the state-of-the-art technology can be used to its full potential. Cell sorting is generally performed by an FCF operator, while cytometric analyses are carried out by the researchers. To assure a high standard, the FCF staff is providing a sound user training, regular maintenance and calibration of the equipment and correct sample handling. The FCF support covers advice on the experimental design (e.g. sample preparation, selection of optimal antibody panel and fluorophores), the proper setup of instruments and advice in data visualization, interpretation and analysis. In addition, the FCF staff is also supporting cytometers and cell sorters at DBM-Mattenstrasse and DBM-Petersplatz and is providing technical advice to their researchers.

Infrastructure

The FCF equipment offers the possibility to detect up to eighteen different fluorescent tags to define specific cell types, which is providing an unparalleled, multiparametric tool for cell characterization and cell isolation. Currently, the facility is maintaining an equipment park with two BD FACSAria III and two BD Influx cell sorters, as well as three high-end BD Fortessa flow cytometers, three Beckman Coulter CytoFlex and two BD Accuri C6. The facility is accessible to trained users on a 24h/365d basis.

While the core facility staff is following the latest technological developments, a steering committee of DBM research group leaders is providing instrumental guidance. This allows the facility to swiftly adapt to the evolving needs of the DBM research community.

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DBM Mattenstrasse

Researcher working on a flow cytometer in the Flow Cytometry Facility.

Joint Core Facilities

Joint Core Facilities not only provide services to researchers at the DBM, but also to other life sciences institutions in the Basel area.
They are jointly managed by the contributing partners and form an important pillar in assuring access to state of the art infrastructure and technical expertise to our research groups while keeping up with the rapid technological developments.

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Genomics Facility Basel

Providing a central platform for next generation sequencing technologies and genomics research.

The Genomics Facility Basel is a joint venture between the Department of Biosystems Sciences and Engineering (D-BSSE) of ETH Zürich and the life sciences departments of the University of Basel. They provide professional expertise and technical support for all steps in the NGS workflow, including library preparation, (single-cell) RNA-seq, genome and exome sequencing and the development of new protocols and applications. The Genomics Facility Basel works in close collaboration with the DBM Bioinformatics Core Facility.

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www.biozentrum.unibas.ch/research/groups-platforms/overview/unit/lstf/

Life Sciences Training Facility (LSTF)

Understanding gene function in health and disease.

The Life Sciences Training Facility (LSTF) is a technology platform, which is supported by the Department Biozentrum, the Transfaculty Research Platform Molecular and Cognitive Neurosciences and the Department of Biomedicine. The LSTF provides access to deep-sequencing and microarray technologies and contributes to the identification of novel molecular pathways. The LSTF works in close collaboration with the Genomics Facility Basel.

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Academic head
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Small Animal Facility for Preclinical Nuclear Molecular Imaging

In vivo imaging of radiolabelled compounds at high sensitivity.

The facility is operated in collaboration with the Divisions of Radiopharmaceutical Chemistry and Nuclear Medicine at the University Hospital Basel. It provides access to SPECT/CT technology for short- and long-term experiments in living rodents and other equipment for the analysis of pharmacokinetic and –dynamic properties of radiolabeled compounds. The different nuclear imaging modalities allow highly sensitive detection of molecules in the femtomolar range in an endogenous setting – a prerequisite to avoid interfering interactions such as receptor saturation effects.

Center for Transgenic Models (CTM)

Designing and generating custom transgenic models.

The CTM at the University of Basel provides researchers access to the latest transgenic and assisted reproduction technologies, while at the same time implementing the 3R principles for responsible animal experimentation. Their services include DNA pronuclear injection, rapid oocyte injection, embryo cryopreservation, mouse strain rederivation, chimera generation and sperm cryopreservation and transgenic model design. The CTM also provides technical advice on TALENS and CRISPR technology and is developing novel gene delivery techniques.

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BioEM Lab at Center for Cellular Imaging and Nano Analytics (C-CINA)

Electron microscopy for the life sciences.

The BioEM Lab of the University of Basel gives researchers access to electron microscopy structural investigations in the life sciences field. The BioEM Lab offers various life sciences high-resolution electron microscopy imaging methods for cellular EM (e.g. TEM imaging and volume reconstructions of tissues) and molecular EM (e.g. high-resolution cryo-EM 3D reconstructions of biological macromolecules). By providing training, consulting and technical support, the facility staff supports researchers in defining their workflow for sample preparation, data acquisition and image processing.

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Center for Scientific Computing (sciCORE)

Facilitating access to high-performance computing infrastructure.

The sciCORE at the University of Basel is maintaining and developing high-performance computing infrastructures, large scale storage systems and an up-to-date software stack. sciCORE staff provides training, consulting and direct support to the researchers in the use of these resources and is actively participating in computational projects of national scope. At the DBM, the Bioinformatics Core Facility is closely interacting with sciCORE to provide the researchers optimal access to the required resources.

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RESEARCH GROUPS

Neurobiology

Bernhard Bettler
Department of Biomedicine
Physiology
University of Basel

Ludwig Kappos
Department of Biomedicine
Division of Neurology
University Hospital Basel

Understanding the molecular events underlying diseases of the nervous system and exploiting this knowledge for improving treatment are among the major challenges in the life sciences. In view of the increasing social and financial burden generated by these diseases, especially in the setting of an ageing population, the Department of Biomedicine (DBM) has defined the neurosciences as one of its focal areas.

The Focal Area Neurobiology of the DBM complements parallel efforts at the Biozentrum and at the Friedrich Miescher Institute (FMI) and is part of the Neuroscience Network Basel (NNB), which was acknowledged as a center of competence by the University of Basel in fall 2008. The NNB follows a translational strategy and comprises more than 400 neuroscientists from 40 different laboratories associated with the University, the University Hospitals, the FMI and the Basel Life Science Industry.

Research is conducted at all levels – from molecules to behavior – thus providing outstanding research opportunities and an excellent platform for a strong educational program. Furthermore, the NNB offers weekly research seminars and lecture series at the graduate and postgraduate levels, covering all aspects of basic and clinical neuroscience. Finally, the NNB is part of the trinational educational and collaborative NEUREX network along with the neuroscience programs at the Universities of Freiburg (Germany) and
Strasbourg (France).

A major aim of the Neuroscience groups at the DBM is to take advantage of the unique expertise in the neurosciences present in the Basel area to pursue translational research projects. As a consequence of these efforts, basic and clinical neuroscientists have contributed to the development of innovative therapies with leading roles in international colaborations and successfully raised grant support for translational research projects from the Swiss National Science Foundation, the European Union, the Swiss Cancer League, the Swiss MS Society and various private foundations. The focus of these projects is on neuroinflammatory, neurodegenerative, psychiatric, neurological and neuromuscular disorders. Several members of the DBM/NNB are actively involved in the National Centre of Competence in Research (NCCR) “The Synaptic Bases of Mental Diseases”.

To promote the rapid translation of research results into clinical practice the DBM Focal Area Neurobiology co-organizes the Annual Basel Neuroscience Symposium “From Bench to Bedside”. The one-day event provides a platform for exchange of ideas and is regularly attended by more than 150 local neuroscientists, including basic and clinical researchers from Novartis, Roche, Actelion, Santhera Pharmaceuticals, the FMI and the University of
Basel.

Molecular Neurobiology Synaptic Plasticity

Bernhard Bettler
Department of Biomedicine
Physiology
University of Basel

Group Members

Lisa Adelfinger (PhD Student)*
David Berner (PhD Student)*
Valérie Besseyrias (Technician)
Dr. Margarita Dinamarca Ceballos (Postdoc)
Ramona Felix (Administrative Assistant)
Dr. Thorsten Fritzius (Postdoc)
Dr. Martin Gassmann (Research Associate)
Dr. Anja Harmeier (Postdoc)*
Dr. Txomin Lalanne (Postdoc)
Dr. Alessandra Porcu (Postdoc)*
Dr. Adi Raveh (Postdoc)
Pascal Dominic Rem (PhD Student)
Dr. Yanwei Tan (Postdoc)
Dr. Rostislav Turecek (Postdoc)
Dr. Celine Ullrich (Postdoc)
Dr. Daniel Ulrich (Postdoc)
Dr. Ruth Werthmann (Postdoc)*
Dr. Xiaomo Wu (Postdoc)

*left during report period

Deconstructing and reconstructing signaling complexes of G-protein coupled receptors in the nervous system

The signaling repertoire of G-protein coupled receptors (GPCRs) in the central nervous system is expanded through interaction with trafficking, effector and regulatory proteins that constitute stable multi-protein receptor complexes with distinct composition and localization. We have started to deconstruct and reconstruct GPCR complexes in native and recombinant expression systems to address how the composition of such complexes influences fundamental processes such as neuronal excitability and network oscillations. Our ultimate goal is to identify novel drug targets for the treatment of neurological and psychiatric diseases.

$GABA_B$ receptors

$GABA_B$ receptors are the GPCRs for the inhibitory neurotransmitter γ-aminobutyric acid (GABA). $GABA_B$ receptors have been implicated in a variety of neurological and psychiatric conditions, including epilepsy, essential tremor, anxiety, depression, schizophrenia, bipolar disorder, addiction and pain. Despite the involvement of $GABA_B$ receptors in mental health disorders, the clinical use of $GABA_B$ receptor drugs is currently limited to agonists and the treatment of narcolepsy, neuropathic pain, spasticity and dystonia. One reason for this is that the main therapeutic effect of baclofen – muscle relaxation – is an unwanted side effect for mental health indications.

A large body of work supports that native $GABA_B$ receptors vary in their kinetic and signaling properties. To some extent, this may be explained by receptor-associated proteins. In collaboration with B. Fakler (Freiburg iBr) we purified native $GABA_B$ receptor complexes and identified ~30 constituents using tandem mass-spectrometry (Schwenk *et al.*, Nature Neurosci., 2016). We found that $GABA_B$ receptors not only comprise principal $GABA_{B1}$ and $GABA_{B2}$ subunits but also four auxiliary KCTD subunits. These auxiliary subunits regulate the kinetics of the receptor response and influence circadian rhythmicity and emotions in behaving mice (Turecek *et al.*, Neuron, 2014; Cathomen *et al.*, Transl. Psychiatry, 2016). Principal and auxiliary receptors subunits, together with the heterotrimeric G-protein, can be viewed as the core building blocks of receptors. Peripheral building blocks can bind to these core building blocks and assemble into receptor complexes with unique properties. Peripheral building blocks comprise effector channels, elements of the presynaptic release machinery and proteins that regulate receptor localization. For example, we identified HCN channels (Schwenk *et al.*, Nature Neurosci., 2016) and TRPV1 channels (Hanack *et al.*, Cell, 2016; collaboration with J. Siemens, Heidelberg) as novel effector channels of $GABA_B$ receptors. Together with H. Bräuner-Osborne (Copenhagen), a medicinal chemist, we are currently developing synthetic compounds that interfere with defined receptor complexes. These compounds will be tested for their therapeutic potential in psychiatric indications. The advantages of such compounds could include a reduction in side effects as well as entirely new therapeutic applications.

mGlu5 Receptors

In collaboration with L. Lindemann (Roche, Basle) we have identified mGlu5 receptor-associated proteins. We are currently characterizing the newly identified proteins for their effects on mGlu5 receptor functions *in vitro* and *in vivo*.

Dopamine Receptors
In collaboration with B. Fakler (Freiburg iBr.) and C. Lüscher (Geneva) we have been awarded a Sinergia grant from the Swiss National Science Foundation to identify dopamine receptor-associated proteins. We are analyzing several receptor-associated proteins for their effects on dopamine receptor functions *in vitro* and *in vivo*.

Trace Amine Receptor 1
In collaboration with M. Höhner (Roche, Basel) we have found that TAAR1 activation silences GSK3β signaling of TAAR1/dopamine D2 receptor complexes (Harmeier *et al.*, European Neuropsychopharmacol., 2015). Given that patients with schizophrenia or bipolar disorder exhibit increased GSK3β signaling, TAAR1 deserves consideration as a target for the treatment of psychiatric disorders.

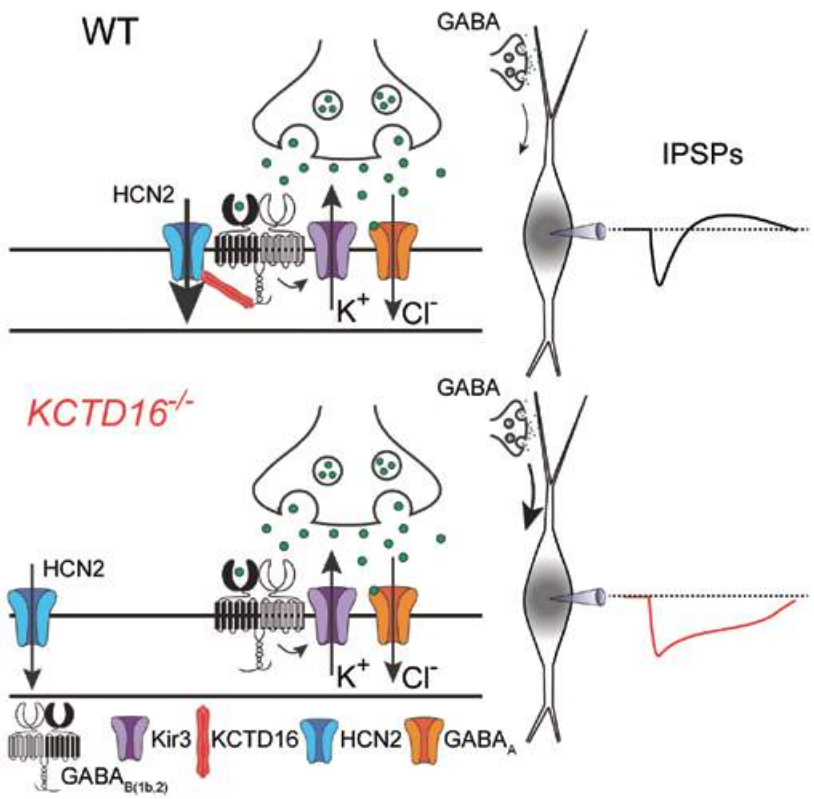

Fig. 1: Scheme depicting the functional consequences of the association of $GABA_B$ receptors and hyperpolarization-activated cyclic nucleotide-gated HCN2 channels in WT but not in *KCTD16*$^{-/-}$ dopaminergic neurons. Proximity of HCN2 channels and $GABA_B$ receptors at the postsynapse of GABAergic synapses in WT neurons facilitates activation of HCN2 channels by hyperpolarizing GABA-mediated IPSPs (induced by $GABA_B$ receptor-activated Kir3-type K^+ channels and $GABA_A$ Cl^- channels). Activated HCN2 channels introduce an additional shunt in the membrane that interferes with propagation of IPSPs to the soma of dopaminergic neurons (indicated with a weak arrow). In *KCTD16*$^{-/-}$ neurons HCN2 channels are dissociated from receptors and their effector Kir3 channels, which removes HCN2 channels from the hyperpolarizing influence of IPSPs. A drift of the HCN2 channels to more distal dendritic locations, relative to the postsynapse, results in stronger signal propagation and larger IPSPs at the soma (indicated with a strong arrow). Adapted from Schwenk *et al.*, Nature Neurosci. 19, 2016.

Connection to Clinical Practice

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Neural Stem Cells and Notch Signaling in Brain Tumors
We collaborated with M. Sailer and R. Gutzmann on a neural stem cell culture model that can be used to investigate the epithelial to mesenchymal transition (Sailer *et al.*, Jove, 2016). We further collaborated with V. Taylor, L. Mariani , S. Frank on a tumor suppressor function for Notch signaling in brain tumors (Giacchino *et al.*, Cancer Cell, 2015).

Selected Publications

Pin J-P, Bettler B. (2016). Organization and functions of mGlu and GABAB receptor complexes. Nature 540(7631), 60–68

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Zhang J, Tan L, Ren Y, Liang J, Lin R, Feng Q, Zhou J, Hu F, Ren J, Wie C, Yu T, *et al.* (2016). Presynaptic Excitation via GABAB Receptors in Habenula Cholinergic Neurons Regulates Fear Memory Expression. Cell 166(3), 716-28

Turecek R, Schwenk J, Fritzius T, Ivankova K, Zolles G, Adelfinger L, Jacquier V, Besseyrias V, Gassmann M, Schulte U, *et al.* (2014) Auxiliary GABAB receptor subunits uncouple G-protein βγ subunits from effector channels to induce desensitization. Neuron 82, 1032–1044

Hanack C, Moroni M, Lima WC, Wende H, Kirchner M, Adelfinger L, Schrenk-Siemens K, Tappe-Theodor A, Wetzel C, Kuich H, *et al.* (2015) GABA blocks pathological but not acute TRPV1 pain signals. Cell 160(4), 759–770

Cellular Neurophysiology

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Synaptic integration of young neurons into the adult hippocampus

The hippocampal formation within the medial temporal lobe of the cerebral cortex is essential for our conscious memory for facts and events. Remarkably, the hippocampus is one of the very few regions in the adult mammalian CNS, where new neurons are continuously generated throughout life. This indicates that the new neurons are involved in learning and formation of new memories. However, the underlying mechanisms are still poorly understood.

During the last three years (2014–2016) we have investigated the regulation of adult neurogenesis on a cellular and behavioural level, and investigated the effects of enhanced neurogenesis on learning and memory in adult mice. Within the hippocampus, neurogenesis is restricted to granule cells in the dentate gyrus (Figure 1). Adult neural stem cells (green) give rise to transit amplifying cells (yellow) generating postmitotic neuroblasts which subsequently differentiate into young neurons (red). During the following ~6 weeks, they form new dendrites and several thousand new synaptic connections within the adult hippocampal network.

We investigated the properties of the first GABAergic and glutamatergic synapses which sequentially appear a few days and about 2 weeks post mitosis, respectively (Heigele *et al.* 2016, Fig. 1). To investigate adult neurogenesis, we have used an oncoretrovirus which inserts into the DNA of dividing cells to label newly generated neurons with GFP which enables analysis of the young cells in hippocampal brain slices. As a second approach, we use transgenic mice expressing the red fluorescent protein DsRed under the control of doublecortin (DCX-DsRed) which is expressed in young neurons for about 3 weeks post mitosis (Fig. 2).

Although GABA is an inhibitory transmitter in the adult brain, we have shown that GABA is depolarizing (EGABA= -35 mV) in the newborn neurons for a period of about 3 weeks (Heigele *et al.* 2016). After 3 weeks the GABA reversal potential shifts to more negative values close to the granule cells resting potential to finally act inhibitory in mature neurons. As glutamatergic synapses are very sparse and inefficient until about 3 weeks post mitosis it was believed that the young neu-

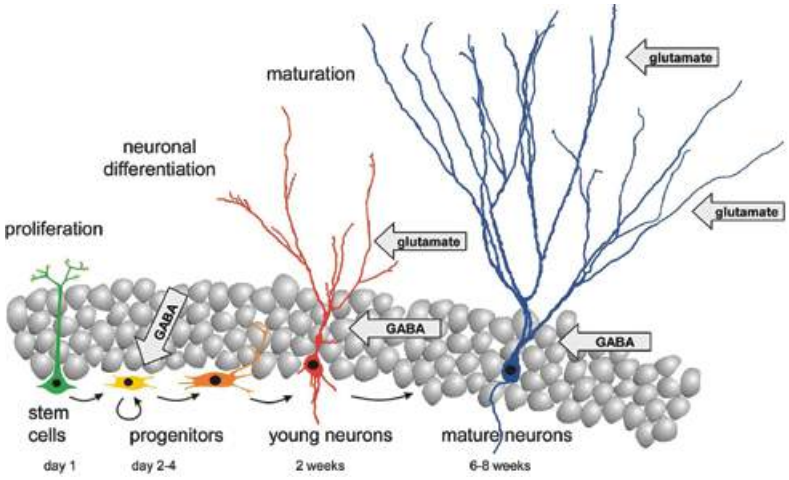

Fig. 1: Adult neurogenesis in the hippocampus. Adult neural stem cells are localized within the subgranular zone of the hippocampus to generate neural progenitors and thereby postmitotic neurons. During a period of about 6–8 weeks the surviving neurons form thousands of new GABAergic and glutamatergic synaptic contacts and fully integrate into the mature hippocampal network.

Fig. 2: Newborn neurons in adult mice. A horizontal hippocampal brain slice was prepared from a 2-month-old transgenic mouse expressing the red fluorescent protein DsRed und the control of the doublecortin (DCX) promotor, labeling young neurons form 1–3 weeks post mitosis. Newborn granule cells are localized within the inner border of the granule cell layer. Whereas DsRed is localized in the soma (red), the DCX protein is distributed along soma, dendrites and axons shown by immunolabelling (green). Neurogenesis was increased by keeping animals for 2–3 weeks in cages with running wheels (see Fig. 3).

rons do not fire synaptically evoked action potentials (APs). Remarkably, we could show that depolarizing GABAergic synapses can evoke or inhibit APs in the young cells, dependent on the number of active presynaptic GABAergic interneurons. Low GABAergic conductances (1–2 nS) corresponding to about 3–4 interneurons, reliably evoked action potentials. By contrast, when more than 30 % of the synaptically connected interneurons were active (> 4 nS), spiking was blocked via shunting inhibition. Therefore, moderate interneuron activity could activate young neurons and thereby promote functional maturation and synaptic integration of the young cells.

In a second project we have studied the formation of new glutamatergic synapses at 2–4 weeks post mitosis and its regulation by local astrocytes in collaboration with Nicolas Toni (Sultan *et al.* 2015). Astrocytes release D-serine in an activity-dependent manner, which is an important cofactor to activate synaptic and extrasynaptic NMDA receptors, abundantly expressed in the young neurons during the period of synapse formation (Schmidt-Salzmann *et al.* 2014). Therefore, astrocytes are critically important to regulate new synapse formation in an activity-dependent manner.
It is well known that physical activity is associated with hippocampal network activity. Therefore, we investigated the consequences of 2–3 weeks of voluntary wheel running in adult mice (Figure 3, Bolz *et al.* 2016). We studied the number of newborn neurons and episodic memory using a "novel object recognition task". Furthermore, we have used different types of objects which were either very similar or highly distinct to each other. Whereas both, control and running mice could remember different distinct objects after the 24h delay period, only the running mice could faithfully recognize the different similar objects (Figure 3D), indicating that increased neurogenesis is in particular important for distinction of similar memory items during memory recall.

Selected Publications

Heigele S, Sultan S, Toni N, Bischofberger J. (2016) Bidirectional GABAergic control of action potential ring in newborn hippocampal granule cells. Nat Neurosci. 19:263–70

Sultan S, Li L, Moss J, Petrelli F, Cassé F, Gebara E, Lopatar J, Pfrieger FW, Bezzi P, Bischofberger J, Toni N. (2015) Synaptic Integration of Adult-Born Hippocampal Neurons Is Locally Controlled by Astrocytes. Neuron 88:957–72

Bolz L, Heigele S, Bischofberger J. (2015) Running improves pattern separation during novel object recognition. Brain Plasticity 1:129–141

Giachino C, Barz M, Tchorz JS, Tome M, Gassmann M, Bischofberger J, Bettler B, Taylor V. (2015) GABA suppresses neurogenesis in the adult hippocampus through GABAB receptors. Development 141:83–90.

Schmidt-Salzmann C, Li L, Bischofberger J. (2014) Functional properties of extrasynaptic AMPA and NMDA receptors during postnatal hippocampal neurogenesis. J Physiol. 592:125–40

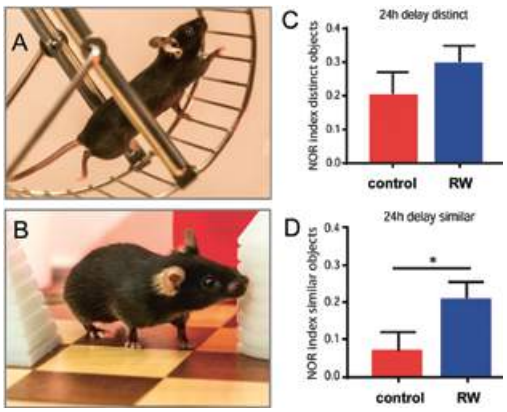

Fig. 3: Running increase pattern separation during novel object recognition. **(A)** Adult mice were kept either in control cages (control) or in cages with running wheels (RW) for 2–3 weeks. **(B)** Afterwards, the animals had to recognize and distinguish previously explored familiar and novel objects. **(C)** Both, running and control mice could recognize different distinct objects, briefly explored 24h before. **(D)** Only running mice (RW), however, could reliably recognize different similar objects. Recognition was measured as differential exploration time, quantified as "novel object recognition" (NOR) index.

Brain Ischemia and Regeneration

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Therapeutic relevance of neural stem cells for white matter regeneration in neonatal hypoxic-ischemic brain injury

Neonatal hypoxic-ischemic (HI) brain injury is the result of an impaired delivery of oxygen and/or blood to the infant's brain. One of its typical hallmark is white matter damage, which can severely affect the development of the brain, leading to devastating sensory-motor, cognitive and learning deficits in the growing child. Currently no available therapy targets the long-term consequences of early brain injury, making regenerative medicine a promising therapeutic option. Several reports suggest that transplanted neural stem cells (NSCs) promote CNS tissue repair not only through cell replacement, but also by providing trophic and immunomodulatory support for endogenous repair mechanisms, including neurogenesis and oligodendrogenesis. A major focus of our research aims at investigating the cellular and molecular mechanisms underlying the neuroprotective role of transplanted NSCs in the context of developmental brain injuries.

Impact of NSCs on oligodendrocyte progenitor cells (OPCs)
We have promising preliminary data showing that endovascular injection of human embryonic stem cell (ESC)-derived NSCs improve both sensory-motor and cognitive functions in a rodent model of neonatal HI. We observed that NSCs treatment specifically stimulates white matter repair mechanisms such as OPCs proliferation and maturation with significant increase in myelin basic protein expression. Nevertheless, the specific molecular mechanism by which NSCs exert this beneficial effect on OPCs are currently unknown. To address this issue, we are performing *in vitro* experiments testing the impact of the conditioned medium from human NSCs onto OPCs cultures (Fig. 1) as well as direct cellular interaction between NSCs and OPCs. Our objective is to identify the specific trophic factors released by NSCs that can influence the oligodendroglial lineage.

Interaction between microglia and NSCs
NSC-mediated regeneration may also occur through immunomodulation of microglia, the immune cells of the brain. Indeed, our research points to a direct interaction between NSCs and microglia through NSC-secreted factors *in vitro* and *in vivo*, in the healthy animals, as published in Mosher *et al.,* 2012. To further understand this interaction, we are investigating the impact of a neonatal HI brain injury on the phenotype of microglia in the subventricular zone (SVZ), one of the neurogenic niches (Fig. 2). The hypothesis is that SVZ microglia adopt a pro-neurogenic phenotype, which might contribute to CNS regeneration following HI. Our histological data show that the phenotype of microglia undergoes dramatic changes postnatally, and that the HI insult impacts durably these physiological changes. Transcriptomic analyses indicate that HI-exposed SVZ microglia adopt a complex phenotype resembling that observed in neurodegenerative diseases, an intriguing finding that merits further investigation. The implications for SVZ neurogenesis *in vivo* remain to be explored.

Developing matrices to optimize NSCs survival into the host tissue
NSCs are typically delivered in a medium solution such as saline. However, once injected, transplanted NSCs show limited survival into the host tissue, thus significantly shortening their therapeutic time-window. To overcome this issue, NSCs can be incorporated in biocompatible matrices. The advantage of such matrices is that they provide physical support for the NSCs, and they can be engineered to carry trophic factors. This research aims at developing matrices that ameliorate NSCs survival, and eventually potentiate central and peripheral neural tissue regeneration. This work is done in collaboration with Prof. D. Kalbermatten and Dr. S. Madduri.

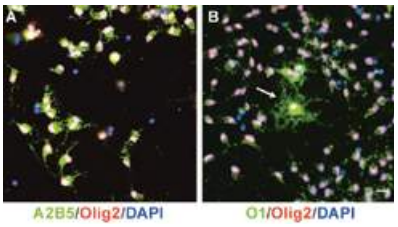

Fig. 1: *In vitro* **maturation of oligodendrocyte progenitor cells (OPCs).**
Representative 20 X photomicrographs of enriched cultures of OPCs isolated from 2-days old rat neonates. (A) shows OPCs stained with A2B5 (green), a marker of immature OPCs, and Olig2 (red), a nuclear marker of the whole oligodendroglial lineage. (B) shows maturing OPCs labeled with O1 (green), and Olig2 (red). Nuclei are counterstained with DAPI (blue). During maturation, the morphology of the OPCs evolves from a bipolar to a more ramified, complex shape (depicted by the white arrow on B). (Scale = 20 µm)

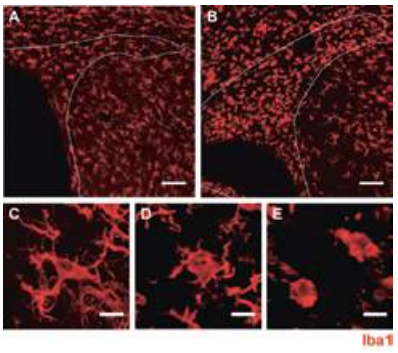

Fig. 2:
Iba1-positive microglia are more numerous, and become activated in the SVZ three days after neonatal HI in rat.
Representative 20X photomicrographs showing Iba1 immunostaining (red) in the SVZ (outlined in white) from sham neonates (A) and in the ipsilateral SVZ from HI neonates (B). Microglial activation is reflected in changes in cellular morphology, from a resting-ramified state (C) to an intermediate (D) and a highly active-amoeboid state (E). (Scale = 100 µm for A and B; scale = 10 µm for C, D and E)

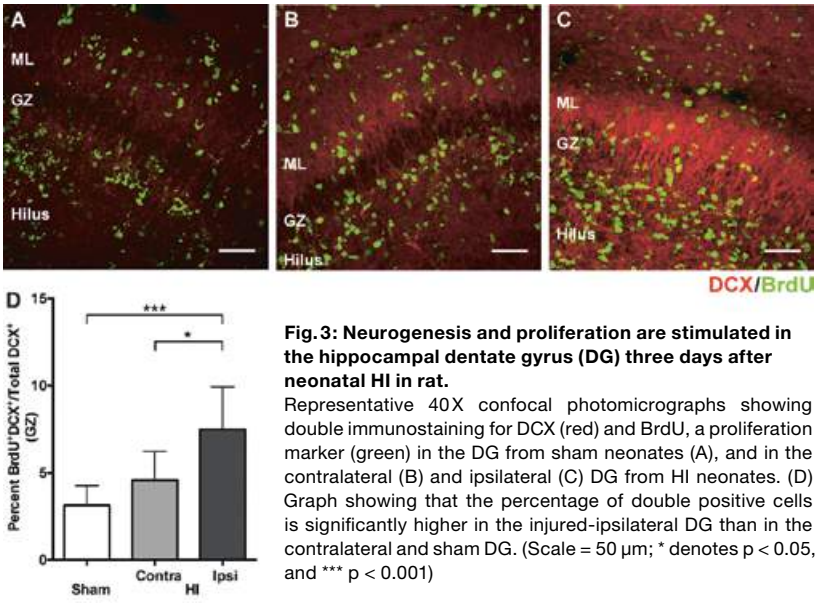

Fig. 3: Neurogenesis and proliferation are stimulated in the hippocampal dentate gyrus (DG) three days after neonatal HI in rat.
Representative 40X confocal photomicrographs showing double immunostaining for DCX (red) and BrdU, a proliferation marker (green) in the DG from sham neonates (A), and in the contralateral (B) and ipsilateral (C) DG from HI neonates. (D) Graph showing that the percentage of double positive cells is significantly higher in the injured-ipsilateral DG than in the contralateral and sham DG. (Scale = 50 µm; * denotes $p < 0.05$, and *** $p < 0.001$)

Our research is funded by the Swiss National Found (SNF) grant 146632 and SNF grant 31003A_163305 in collaboration with the Fetal and Neonatal Stress Research Group of Prof. S. Wellmann at UKBB. The tissue engineering project is supported by a research grant from the Department of Surgery in collaboration with Prof. D. Kalbermatten and Dr. S. Madduri. We have active international collaboration with Stanford University (United States), Newcastle University (England) and the University of Saskatchewan (Canada).

Connection to Clinical Practice

CSF markers of endogenous regenerative processes in the developing brain

Our group teamed up with the CNS Discovery Department of Hoffmann-La Roche to evaluate potential cerebrospinal fluid (CSF) biomarkers with a predictive value for neurodevelopmental impairments, as there is currently an unmet clinical need for such markers. One of our candidate biomarkers was doublecortin (DCX), a CNS protein considered as a marker of neurogenesis in the brain (Fig. 3). Through the use of a novel immunoassay (developed by Roche) that allows quantification of doublecortin, we examined the relevance of this protein in the CSF, using the rat model of neonatal HI. Our investigations revealed that doublecortin in the CSF was both a reflector of stroke severity and of HI-induced neurogenesis. We are currently exploring the translational value of these findings by analyzing doublecortin and other CNS biomarkers in the CSF of pediatric patients.

Selected Publications

Rosenblum S, Smith TN, Wang N, Chua JY, Westbroek E, Wang K, and Guzman R. (2015) BDNF pretreatment of human embryonic-derived neural stem cells improves cell survival and functional recovery after transplantation in hypoxic-ischemic stroke. Cell Transplant 24, 2449–2461.

Chicha L, Smith T and Guzman R. (2014) Stem cells for brain repair in neonatal hypoxia-ischemia. Childs Nerv Syst 30, 37–46

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Developmental Neurobiology and Regeneration

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Purkinje cell dendritic development and vascular plasticity in the Central Nervous System

The dendritic tree reflects the neuronal cell type and the synaptic connections of a given neuron. During development the shape of dendrites is determined by outgrowth and retraction of dendritic processes. Our laboratory is interested in how neuronal activity may be linked to the shaping of the dendritic tree of Purkinje cells, the principal cells of the cerebellar cortex. The dendritic tree of Purkinje cells is well developed in organotypic slice cultures (Fig. 1). In previous work we have shown that glutamate receptor activation and increased protein kinase C activity strongly inhibit dendritic development of Purkinje cells. Their inhibitory action is mediated by changes in the calcium equilibrium of the Purkinje cells. The control of intracellular calcium is an important link between functional activity and dendritic growth in Purkinje cells we want to further elucidate how changes in functional activity are converted to changes in dendritic growth.
For the long-term control of the calcium equilibrium in Purkinje cells calcium clearing mechanisms are essential. We have investigated the role of the plasma membrane Ca2+- ATPase 2 (PMCA2) which is highly expressed in Purkinje cell dendritic spines. When we applied chronic pharmacological inhibition of PMCA2 function with carboxyeosin we found a mild reduction of the size of Purkinje cell dendritic trees similar to the observation in PMCA2-deficient mice. However, when PMCA2 inhibition was combined with mGluR stimulation it had a partial rescuing effect similar to that seen after blockade of voltage-gated calcium channels. We are currently studying the role of the sodium calcium exchanger (NCX), another important calcium clearing mechanism in Purkinje cells. In addition to controlling dendritic development, protein kinase C activity in Purkinje cells is also linked to SCA-14, a hereditary cerebellar disease with cerebellar dysfunction and Purkinje cell degeneration. SCA-14 is a particular interesting model because its phenotype and clinical settings are indistinguishable from other types of SCA which are caused by polyglutamine expansions. SCA-14, in contrast, is caused by point mutations in the coding region of the PKCgamma gene. The mechanisms by which the mutations in the PKCgamma gene lead to Purkinje cell degeneration in SCA-14 are still rather unclear. We have generated a transgenic mouse model in which a mutated form of protein kinase C gamma is expressed specifically in Purkinje cells. In the human disease this mutation causes Purkinje cell dysfunction, degeneration and death. We could show that the presence of the mutated protein in mouse Purkin-

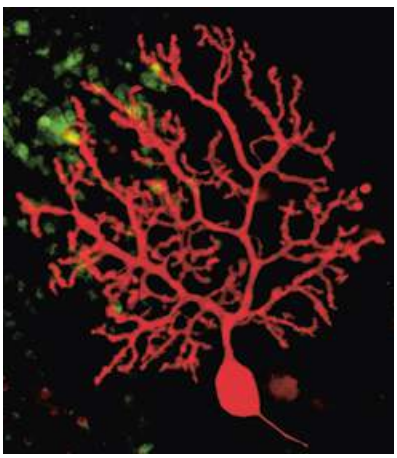

Fig. 1: View of a Purkinje cell in an organotypic slice culture after 12d *in vitro*. Anti-calbindin staining for Purkinje cells is shown in red. The elaborate dendritic tree of this cell has developed almost entirely during the culture period.

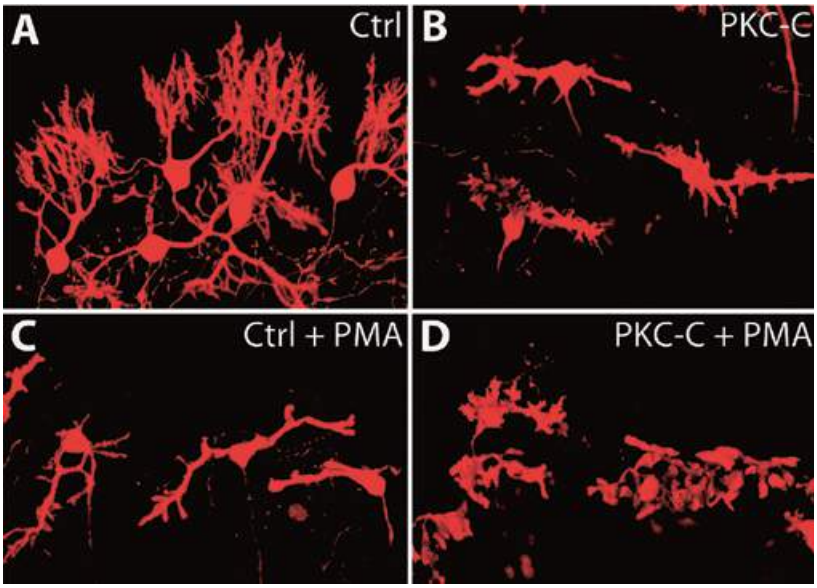

Fig. 2: The Purkinje cell dendritic tree in slice cultures derived from mice with transgenic expression of a mutated PKCgamma from SCA14 (B) is severely compromised compared to control (A) and resembles strongly that of Purkinje cell with pharmacological activation of PKCgamma (C). Further pharmacological activation of PKCgamma in mutant Purkinje cells results in only a minor additional reduction of the dendritic tree (D). Modified from Ji *et al.* 2014.

Selected Publications

Shimobayashi E, Wagner W, Kapfhammer JP. (2015) Carbonic Anhydrase 8 Expression in Purkinje Cells Is Controlled by PKCγ Activity and Regulates Purkinje Cell Dendritic Growth. Mol. Neurobiol. [Epub ahead of print], doi: 10.1007/s12035-015-9444-3

Ji J, Hassler ML, Shimobayashi E, Paka N, Streit R, Kapfhammer JP. (2014) Increased protein kinase C gamma activity induces Purkinje cell pathology in a mouse model of spinocerebellar ataxia 14. Neurobiol. Dis. 70: 1–11, doi: 10.1016/j.nbd.2014.06.002

Chip S, Zhu X, Kapfhammer JP. (2014) The Analysis of Neurovascular Remodeling in Entorhino-hippocampal Organotypic Slice Cultures. J. Vis. Exp., e52023, doi: 10.3791/52023

Sherkhane P, Kapfhammer JP. (2013) The plasma membrane Ca2+-ATPase2 (PMCA2) is involved in the regulation of Purkinje cell dendritic growth in cerebellar organotypic slice cultures. Neural Plast. 2013: 321685

Chip S, Nitsch C, Wellmann S, Kapfhammer JP. (2013) Subfield-specific neurovascular remodeling in the entorhino-hippocampal-organotypic slice culture as a response to oxygen-glucose deprivation and excitotoxic cell death. J Cereb Blood Flow Metab. 33:508–518

je cells induces a striking reduction of Purkinje cell dendritic outgrowth in organotypic slice cultures indicating that the mutated protein in the Purkinje cells acts like a constitutive active kinase and is biologically active in the Purkinje cells (Fig. 2). Due to the Purkinje cell-specific expression of the transgene we have taken advantage of this mouse model for identifying molecules signalling downstream of increased PKCgamma activity. Using a gene chip array approach we have identified several potential candidate molecules with increased mRNA expression in Purkinje cells. Out of those we have further studied carbonic anhydrase 8 (CA8) and have identified it as a potential mediator of PKCgamma activity and Purkinje cell dendritic development (Fig. 3). Our studies have provided a better understand Purkinje cell dysfunction and may be helpful for developing novel therapeutic strategies for cerebellar diseases.

Neuronal survival is also dependent on an intact blood supply and an intact blood brain barrier. Our group has analysed neurovascular interactions in a slice culture setting. This has allowed us to challenge neurons in organotypic slice cultures by ischemia or excitotoxic compounds and study neuronal survival and the blood vessel architecture simultaneously under these conditions. Our work so far has revealed complex interactions between neuronal survival and maintenance of blood vessels under ischemic conditions. This work has provided novel insights in the yet rather poorly explored neurovascular interactions.

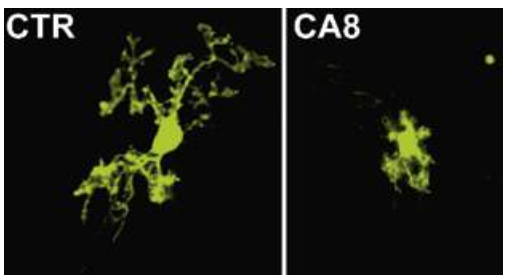

Fig. 3: For transfection studies Purkinje cells were grown in dissociated cultures. After transfection with a control GFP plasmid Purkinje cells develop a typical dendritic tree in such cultures (A). In contrast, after transfection with CA8, the dendritic tree is smaller and dendritic development is compromised (B) indicating that CA is involved in controlling Purkinje cell dendritic growth. Modified from Shimobayashi *et al.* 2015.

Psycho-pharmacology Research

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*left during report period

Pharmacology of novel psychoactive substances, MDMA, and LSD

Our research focus is on the pharmacology of psychoactive substances *in vitro* and in humans. We characterize novel substances (designer drugs) *in vitro* and also investigate the pharmacokinetics-pharmacodynamics of MDMA, LSD, and amphetamines in humans including psychological tests, pharmacogenetics, and functional brain imaging. This work is linked to studies on the acute clinical problems associated with the recreational drug use. In the laboratory, we mainly characterize the receptor interaction profiles of novel psychoactive substances and their cytotoxic effects.

Novel psychoactive substances

Novel psychoactive substances are newly used designer drugs ("internet drugs", "research chemicals", "legal highs") potentially posing similar health risks to classic illicit substances. In the last years we have seen an unprecedented growth in the number of new psychoactive substances on the illicit drug market. Currently, more than one new substance is identified in Europe every week. Information on the pharmacology and toxicology of these substances is important to reduce risks to the public. Many novel psychoactive substances interact with biogenic amine neurotransmitter transporters. Amphetamines including methamphetamine and MDMA inhibit the dopamine, serotonin and norepinephrine transporter and also release these monoamines through the respective transporter. Substances which predominantly release serotonin, similar to MDMA, can be expected to produce MDMA-like effects with serotonergic toxicity including serotonin syndrome, hyponatremia, hyperthermia, and seizures. In contrast, psychostimulants such as methamphetamine or methylphenidate are mostly enhancing dopaminergic neurotransmission. Dopamine mediates the reinforcing and addictive properties of drugs of abuse. The relative dopaminergic to serotonergic properties *in vitro* (dopamine/serotonin transporter inhibition ratio) of a novel substance can be determined as a useful marker for its potential clinical psychotropic and acute toxic effects (Fig. 1).

Mechanism of action of MDMA (ecstasy)

MDMA (ecstasy) acutely induces happiness, emotional empathy, and prosociality. MDMA is used as recreational substance and also investigated as medication to assist psychotherapy in psychiatric patients. We demonstrated a critical role for transporter-mediated serotonin and norepinephrine release in the effects of MDMA in humans. The dual serotonin and norepinephrine transporter inhibitor duloxetine blocked MDMA-induced serotonin and norepinephrine efflux from transmitter loaded cells stably expressing the human serotonin or norepinephrine transporter and prevented the MDMA effects in humans. The findings indicate that the psychotropic effects of MDMA in humans depend on transporter-mediated release of both serotonin and norepinephrine. The response to MDMA is also dependent on interindividual genetically-determined differences in the metabolism of MDMA. For example, subjects who are cytochrome P450 (CYP) 2D6 poor metabolizers show higher plasma concentrations of MDMA and associated faster increases in cardiovascular responses to MDMA compared with subjects who are normal metabolizers (Fig. 2).

Pharmacokinetics of MDMA and LSD and effects on emotion processing

Several clinical studies are investigating the use of MDMA and LSD in substance-assisted psychotherapy to treat post-traumatic stress disorders or anxiety in patients with life-threatening diseases. We therefore studied the pharmacokinetics

of MDMA and LSD and the effects of these substances on the processing of emotions in healthy subjects. LSD produced subjective drug effects that lasted up to 12h (Fig. 3a) and correlated well with the concentrations of LSD in the blood plasma over time (Fig. 3b and c). The half-life of LSD in plasma was 3.5 h. In contrast to LSD, the half-life of MDMA is longer (8h) but the effects of MDMA last only up to 6h despite the continued presence of the substance in the body (Fig. 3d). Thus, there is marked acute tolerance to the effects of MDMA. In a controlled setting, both MDMA and LSD dose-dependently increased feelings of closeness and trust and impaired identification of negative emotions including fear and sadness. These effects of MDMA and LSD on emotion processing may be useful for substance-assisted psychotherapy.

Selected Publications

Simmler LD, Buchy D, Chaboz S, Hoener MC, Liechti ME. (2016) *In vitro* characterization of psychoactive substances at rat, mouse, and human trace amine-associated receptor 1. J. Pharmacol. Exp. Ther. 357, 134–144

Rickli A, Lüthi D, Reinisch J, Buchy D, Hoener MC, Liechti ME. (2015) Receptor interaction profiles of novel N-2-methoxybenzyl (NBOMe) derivatives of 2,5-dimethoxy-substituted phenethylamines (2C drugs). Neuropharmacology 99, 546–553

Rickli A, Kopf S, Hoener MC, Liechti ME. (2015) Pharmacological profile of novel psychoactive benzofurans. Br. J. Pharmacol. 172, 3412–25

Liechti ME. (2015) Novel psychoactive substances (designer drugs): overview and pharmacology of modulators of monoamine signalling. Swiss Med. Weekly, 145, 214043

Schmid Y, Enzler F, Gasser P, Grouzmann E, Preller K, Vollenweider FX, Brenneisen R, Müller F, Borgwardt S, Liechti ME. (2015) Acute effects of LSD in healthy subjects. Biol. Psych. 78, 544–553

Simmler LD, Buser TA, Donzelli M, Schramm Y, Dieu LH, Huwyler J, Chaboz S, Hoener MC, Liechti ME. (2013) Phar macological characterization of designer cathinones *in vitro.* Br. J. Pharmacol. 168, 458–70

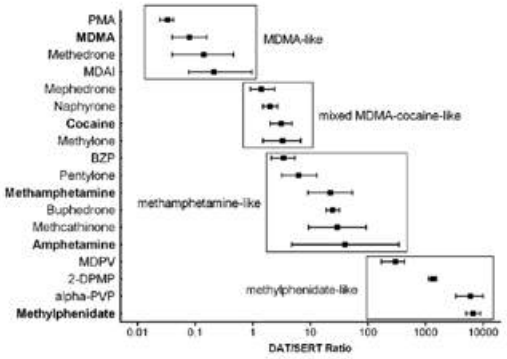

Fig. 1: Relative dopamine/serotonin inhibition potencies of psychoactive substances. Dopamine to serotonin transporter (DAT/SERT) inhibition ratios (mean ± 95% confidence intervals) for novel substances are shown in comparison with those of classic empathogens/entactogens (MDMA, ecstasy) and stimulants (cocaine, amphetamine, and methamphetamine). The ratios derived from *in vitro* studies help to predict the typically unknown clinical toxicity of novel substances. A low DAT/SERT inhibition ratio (<0.1) indicates tenfold greater relative serotonergic vs. dopaminergic activity similar to MDMA. A high DAT/SERT inhibition ratio (>10) indicates greater relative dopaminergic vs. serotonergic activity similar to methamphetamine. A high DAT/SERT inhibition ratio is a pharmacological characteristic associated with more stimulant effects and with higher potential for addiction.

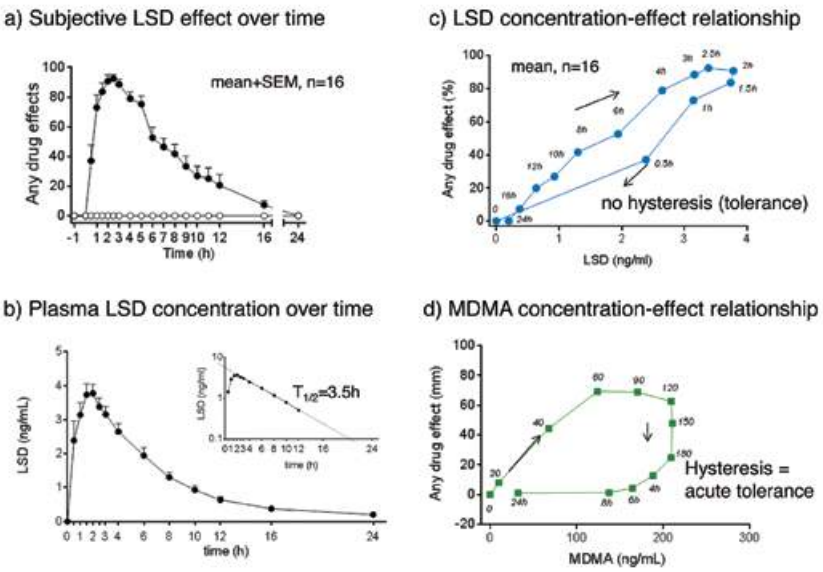

Fig. 3: Pharmacokinetics-Pharmacodynamics of LSD. LSD effects last up to 12h (a) corresponding to its plasma-concentration time curve (b) and exhibiting no hysteresis in the LSD concentration-effect plot (c). In contrast, the MDMA concentration-effect plot shows pronounced hysteresis consistent with acute tolerance (d).

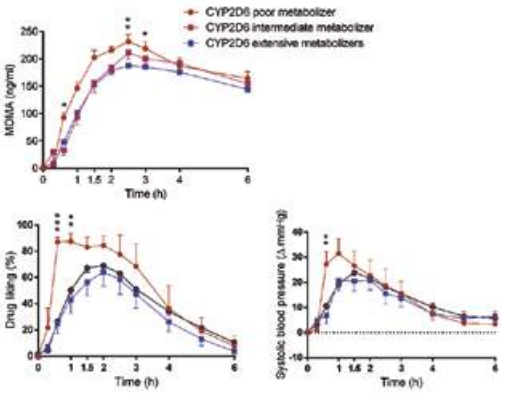

Fig. 2: Pharmacogenetics of MDMA. CYP2D6 phenotypes predicted by genotyping modulate the plasma concentration of MDMA and MDMA-induced increases in blood pressure and subjective drug liking. Efects increased more rapidly in CYP2D6 poor metabolizers (PMs) compared with intermediate (IMs) or extensive metabolizers (EMs). The data are expressed as the mean ± SEM in seven PMs, 19 IMs, and 113 EMs. *P < 0.05, **P < 0.01, ***P < 0.001, PMs compared with IMs or EMs at the corresponding time.

Clinical Neuro-immunology

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Molecular and immunological analysis of Multiple Sclerosis

Our research focuses the molecular and immunological analysis of multiple sclerosis (MS), an inflammatory, demyelinating central nervous system (CNS) disease. We have two main research lines: 1) genomic investigations (including genetic, transcriptional and protein expression analysis) and 2) studies on B cell involvement in MS pathogenesis. Both approaches provide tools and markers for immunomonitoring of current and newly emerging treatments.

Immune regulation by microRNAs in MS

MicroRNAs (miRNAs), small non-coding RNA molecules, which modulate gene-expression of >50% of all protein-encoding genes, and are key regulators of a wide variety of biological processes, e.g. cell proliferation, differentiation, apoptosis and organ development. Our cellular miRNA studies in immune cells from MS patients have revealed distinct expression profiles compared with those in healthy volunteers. We have also shown that natalizumab, the treatment for relapsing-remitting MS, has diverse effects on miRNA expression. We uncovered recently a specific effect of natalizumab on the expression of miR-126 and miR-10 and their potential target, POU2AF1, an important regulator of the transcription factor Spi-B, which binds to unique sequences of the JC virus and plays a critical role in driving virus activity (Meira *et al.*, 2014, 2016, Fig. 1). Natalizumab treatment has been associated with the development of progressive multifocal leukoencephalopathy (PML), a severe opportunistic infection of the CNS caused by reactivation of the latent JC virus. We are presently evaluating the expression of miR-126/10 and POU2AF1 as biomarkers for a PML risk in MS patients treated with natalizumab. Another focus of our research is extracellular miRNAs, stored in extracellular vesicles (EVs), in serum and CSF from MS patients. Our aim is to get new insights into the functional role of EVs in immune regulation and cell-to-cell communication in MS.

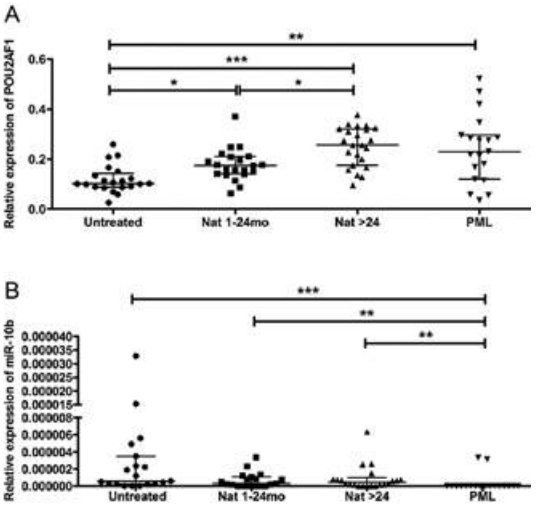

Fig. 1:
Transcriptional expression of POU2AF1 (A) and miR-10b (B) in untreated, natalizumab treated RRMS (1–24mo; >24mo) and natalizumab associated PML patients. Relative expression levels (median with interquartile range) are depicted. ***p<0.001; **p<0.01, *p<0.05.

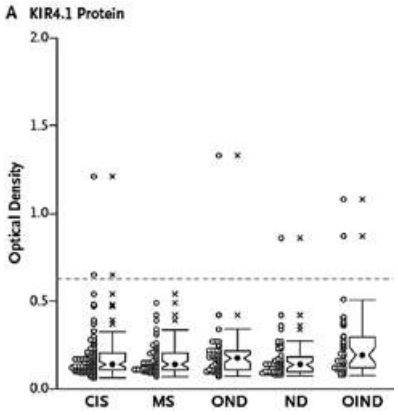

Fig. 2: KIR4.1 protein ELISA: 141 patients with clinically isolated syndrome (CIS, n=82) or multiple sclerosis (MS, n=59) and 131 controls (other (non-inflammatory) neurological diseases (OND) n=48, neurodegenerative diseases (ND) n=48, other inflammatory neurological diseases (OIND) n=35). Anti-KIR4.1 reactivity is expressed as the mean optical density (OD) of duplicate measurements. The distribution of OD KIR4.1 protein by group is shown in notched box-and-whisker plots, each accompanied by histograms of the same data. The dashed horizontal line represents a cut-off value of 0.628 (5 standard deviations (SD) above the mean of an unblinded OND control group1, n=10). Statistical comparison between the groups revealed no significant differences (Kruskal-Wallis rank sum test for five groups: p=0.16).

B cells and their targets in MS

B-cells have a major role in the pathogenesis of MS. Depletion of B-cells leads to a remarkable amelioration of the disease. The mechanisms by which B-cells impact MS are however incompletely understood. Our research focuses on the identification of novel B-cell autoantigens and the characterization of the interaction of autoaggressive B-cells with the CNS. We could show that antibodies against native myelin oligodendrocyte glycoprotein (MOG) identify a subset of patients with neuromyelitis optica (Pröbstel *et al.*, 2015). Antibodies against the potassium channel KIR4.1 have been suggested as a biomarker in MS. Using eukaryotic expression of this protein we could show that the prominently described antibody reactivities are directed against other proteins than KIR4.1 and that the assay as published is not useful for clinical practice (Pröbstel *et al.*, 2016, Fig 2). Currently, we are using transgenic animals to better characterize the pathogenic mechanisms of B cells in the animal model of MS. We identified the co-capture of membrane antigens by the B cell receptor as a key step in initiating an autoimmune response. This work will be continued in analyzing the capacity of B cell to migrate to peripheral tissues and harvest their cognate and non-cognate antigens from the tissue. This phenomenon of membrane capture will also be used to identify novel autoantigens in MS.

Immunomonitoring of new treatments and biomarker research

The mode of action of many current disease modifying treatments is often not well understood. We aim to get a better understanding of the mode of action and thereby identify candidates for treatment response biomarkers. Currently, we are recruiting MS patients that start treatment with dimethyl fumarate. Immune cells of the blood as well as serum will be analyzed for immunological markers, and miRNA/RNA expression profiles that could potentially correlate with the clinical response to treatment. Combination of these biomarkers with a standardized clinical and neuroradiologic assessment will hopefully provide means to better characterize the heterogeneous MS patient populations and to predict the disease course and the treatment response.

Connection to Clinical Practice

Our Laboratory is closely connected to the MS Outpatient Clinic of the Department of Neurology, University Hospital Basel that cares for more than 1000 MS patients per year. This allows access to a population of MS-patients in different stages of the disease. There is also a close collaboration with the Division of Neuroradiology and the Medical Image Analysis Centre (MIAC) that enables characterization of patients with cutting edge neuroimaging techniques. Our Clinical MS Research Group plays a key role in organizing and conducting international therapeutic studies in MS, e.g. with siponimod, amiselimod (Kappos L, 2016), the humanized monoclonal antibodies ocrelizumab, daclizumab, and GNbAC1. These trials provide unique possibilities to apply basic research approaches to understand disease mechanisms and therapeutic responses. Development of biomarkers needs prospective, standardized, and high-quality clinical and neuroradiological data from large patient cohorts to allow for validation and implementation in clinical practice. The Swiss MS Cohort Study (SMSC), supported by the Swiss MS Society and coordinated by our MS Group was initiated in 2012. It aims at building and maintaining a long-term cohort of Swiss MS patients with clinical and MRI follow-up data as well as sampling of body fluids.

Selected Publications

Meira M, Sievers C, Hoffmann F, Derfuss T, Kuhle J, Kappos L, Lindberg RL. (2014) MiR-126: a novel route for natalizumab action? Multiple sclerosis (Houndmills, Basingstoke, England) 20, 1363–1370

Meira M, Sievers C, Hoffmann F, Haghikia A, Rasenack M, Décard BF, Kuhle J, Derfuss T, Kappos L, Lindberg RLP. (2016) Natalizumab-induced POU2AF1/Spi-B upregulation: A possible route for PML development. Neurology® Neuroimmunology & Neuroinflammation 3, e223

Pröbstel A-K, Kuhle J, Lecourt A-C, Vock I, Sanderson NSR, Kappos L, Derfuss T. (2016) Multiple Sclerosis and Antibodies against KIR4.1. New England Journal of Medicine 374, 1496–1498

Pröbstel A-K, Rudolf G, Dornmair K, Collongues N, Chanson J-B, Sanderson NS, Lindberg RL, Kappos L, de Seze J, Derfuss T. (2015) Anti-MOG antibodies are present in a subgroup of patients with a neuromyelitis optica phenotype. Journal of Neuroinflammation 12, 1–7

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Ocular Pharmacology and Physiology

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The optic nerve: Why does it fail and what to do about it?

Loss of retinal ganglion cells (RGCs), responsible for relaying visual information from the retina to the visual centers of the brain, causes vision deficits in about 100 million patients worldwide. Reasons for RGC degeneration are manifold and include genetic mutation and predisposition as well as environmental factors. Recently, the role of the optic nerve microenvironment shaped by meningothelial cells (MECs) became evident with optic nerve compartmentalization connected to optic nerve damage and visual impairment. In addition, mitochondrial dysfunction is also central to RGC failure and death.
Our work focused on characterizing the cellular component of the optic nerve microenvironment and the role of mitochondrial maintenance on neuronal survival. Furthermore, to translate insight into mitochondrial dysfunction towards treatment options for patients suffering from optic nerve degeneration, we are developing artificial transcription factors targeting OPA1, a key regulator of mitochondrial morphology, to treat autosomal dominant optic atrophy.

The optic nerve microenvironment

As part of the central nervous system, the optic nerve is ensheathed by meninges and bathed in cerebrospinal fluid forming a specific microenvironment. Meningothelial cells covering the meninges constitute the cellular component of this microenvironment. Previous work point to an involvement of MECs in pathophysiological processes leading to optic nerve damage as observed during glaucoma. Our recent work revealed, MECs are highly active facultative phagocytes capable of ingesting bacteria and also apoptotic cells, thereby acting immune modulatory by secreting pro- or anti-inflammatory cytokines and chemokines, respectively. Furthermore, MECs are capable of ingesting large amounts of neurotoxic substances, e.g. the Alzheimer's disease-related amyloid-beta peptide, and display a remarkable resistance to such substances. Our work strongly suggests a neuroprotective function for MECs through cerebrospinal fluid conditioning and clearance.

Mitochondrial maintenance and degeneration of neuronal cells

Mitochondrial dysfunction is at the heart of neurodegeneration with specific quality control mechanisms responsible for maintaining mitochondrial and therefore neuronal function. Previously, we studied the role of mitochondrial ubiquitin ligases, now we focused on the AAA-ATPase p97 and its role in mitochondrial maintenacne. We found that p97 is responsible for the removal of oxidatively and nitrosatively damaged mitochondrial proteins and that blockage of this process is deleterious to neuronal cells. We also recently identified three novel co-factors of p97 involved likely involved in maintaining mitochondrial function through targeted protein degradation and targeted autophagic destruction of mitochondria.

Treating autosomal dominant optic atrophy

Dominant optic atrophy (DOA) is a neuro-ophthalmic condition characterized by the degeneration of the optic nerve resulting in blindness. DOA is inherited in an autosomal dominant fashion and is caused by mutations in *OPA1.* OPA1 is a mitochondrial protein and is involved in mitochondrial dynamics and maintenance of mitochondrial cristae structure. Thus, interfering with OPA1 function leads to disturbed mitochondrial dynamics and higher susceptibility to apoptotic stimuli. In DOA, cellular OPA1 levels are reduced due to various haploinsufficient mutations. While this reduction of OPA1 levels to about 50 % does not impact the function of most cells, neurons and especially RGCs depend on full OPA1 function. Thus, restoring wildtype levels of OPA1 in RGCs would constitute a functional cure for DOA.

To this end, we generated a CRISPR-Cas9-based artificial transactivator capable of upregulating *OPA1* in mouse neuronal cells *in vitro* and are in the process of developing viral vectors for delivery of such therapeutics to retinal ganglion cells *in vivo*. In addition, we established two mouse models of DOA in the lab to assess a therapeutic benefit of such artificial CRISPR-Cas9 transactivators for the treatment of this blinding disease.

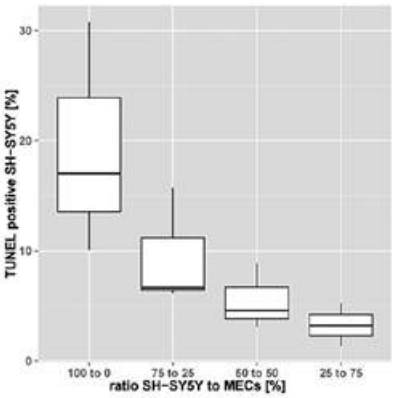

Fig. 1: MECs act neuroprotective by scavenging Aβ. Neuron-like SH-SY5Y cells were co-cultured at identical total density with CellTrace Violet-labeled MECs (Ben-Men-I) at ratios of 100:0, 75:25, 50:50, 25:75 SH-SY5Y:MECs and treated for 24 hours with 25 µM Aβ. TUNEL staining as measure for apoptotic cell death was determined flow cytometrically for CellTrace Violet-negative cells. Shown is the median of three independent experiments as boxplot. Please note the reduction of SH-SY5Y death in the presence of Aβ-scavenging MECs.

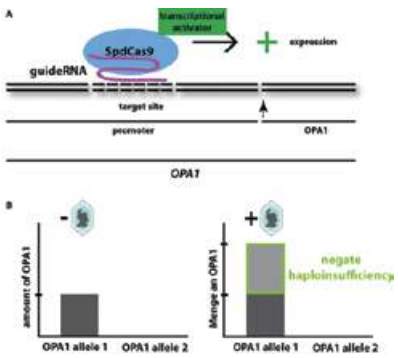

Fig. 2: A novel therapeutic approach for the treatment of dominant optic atrophy. (A) By combining a catalytically inactive form of the RNA-guided *Staphylococcus aureus* endonuclease Cas9 (SpdCas9) with a protein domain capable of driving gene expression (e. g. VP64), a RNA-guided transactivator can be generated. By selecting appropriate guideRNAs, virtually every gene promoter of interest can be targeted. While not all guideRNAs will direct SpdCas9 to a site suitable for inducing gene activation, the relative ease of guideRNA generation allows probing multiple sites inside a gene promoter greatly accelerating development times compared to other artificial transcription factor approaches. **(B)** AAV2-mediated delivery of SpdCas9 to retinal ganglion cells together with a suitable guideRNA targeting the *OPA1*-promoter is envisioned to increase *OPA1* transcript levels, thereby restoring wildtype levels of OPA1 protein, negating haploinsufficiency, and preventing RGC death.

Connection to Clinical Practice

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The ocular pharmacology and physiology lab is closely connected to the University Hospital Eye Clinic under the chairmanship of Prof. Dr. Hendrik Scholl. The main focus is to not only understand mechanisms of (neuro)-degenerative processes of the retina and the optic nerve, but also to modulate these processes to provide better outcomes for patients. In particular, new treatment approaches, including gene therapy and pharmacotherapy, for retinal diseases such as retinitis pigmentosa (RP), Stargardt disease, age-related macular degeneration and diabetic retinopathy are being developed or applied in clinical trials to patients. Also, a close and fruitful collaboration with the Institute of Pathology enables access to valuable tissue samples to study human optic nerves to better understand and validate findings from *in vitro* studies.

Selected Publications

Fang L, Hemion C, Pinho Ferreira Bento AC, Bippes CC, Flammer J, Neutzner A. (2015) Mitochondrial function in neuronal cells depends on p97/VCP/Cdc48-mediated quality control. Front Cell Neurosci. 9, 16

Hemion C, Flammer J, Neutzner A. Quality control of oxidatively damaged mitochondrial proteins is mediated by p97 and the proteasome. Free Radic Biol Med. 75, 121–8

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Li J, Fang L, Meyer P, Killer HE, Flammer J, Neutzner A. Anti-inflammatory response following uptake of apoptotic bodies by meningothelial cells. J Neuroin- ammation. 11, 35

Fang L, Li J, Flammer J, Neutzner, A. MARCH5 inactivation supports mitochondrial function during neurodegenerative stress. Front Cell Neurosci. 7, 176

Neuronal Development and Degeneration

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Propagation of Disease-Linked Proteins in Protein Misfolding Diseases

Our research interest is to understand the role and mechanism of cell-to-cell spreading of toxic proteins in neurodegenerative protein misfolding diseases
In neurodegenerative diseases the nervous system gets progressively altered. Impaired structure and function, which is due to degeneration of neural cells, results in severe behavioral disabilities and often death of the patient.
Protein misfolding diseases (PMDs) are a class of neurodegenerative disorders, in which disease pathogenesis is accompanied by the deposition of β-sheet-rich amyloid aggregates. In each PMD, aggregates are composed of specific misfolded proteins, such as Aβ (amyloid-β) and tau in Alzheimer's disease, α-synuclein in Parkinson's disease, huntingtin in Huntington's disease, superoxide dismutase 1 (SOD1) in amyotrophic lateral sclerosis (ALS) and TAR-DNA binding protein 43 (TDP-43) in ALS and frontotemporal lobar dementia (FTLD) and the prion protein (PrP) in prion disorders. These proteins have little in common, other than showing abnormal folding and aggregation. Pathological lesions in PMDs typically start in disease-specific brain regions from where the pathology progressively spreads throughout the brain in patterns that match functional neuronal connectivity. It was first recognized in prion disorders that this stereotypic spreading is due to transneuronal propagation of the pathogenic form of the PrP (PrP^{SC} or PrP scrapie). A series of exciting new studies have now provided strong experimental evidence that a 'prion-like' self-propagating mechanism is applicable to a variety of disease-linked proteins, including tau, α-synuclein, huntingtin and tdp-43. Furthermore, recent work suggests that the spreading of these pathogenic proteins, like the spreading of pathological lesions, follow disease-specific patterns and neural networks that resemble the architecture of functional synaptic connectivity in the healthy human brain. The spreading of misfolded proteins is therefore likely a common feature of neurodegenerative PMDs and might be an important contributor to non-cell autonomous neuronal damage. However, many aspects of this process remain unknown including its contribution to disease progression and the underlying molecular mechanisms. This hampers the discovery of potential novel therapeutics in PMDs, for which so far no effective treatments exist.
Therefore, the aim of our studies is to understand whether toxic protein spreading is a fundamental trigger for the onset and a key factor for the progression of neurodegeneration. We want to understand the role neuronal connectivity plays in this process and elucidate the underlying cellular/ molecular pathways involved in the spreading of toxic proteins. Initially Huntington's disease is used as a model disease, but the ultimate goal is to know whether toxic protein spreading is a disease pathway shared by Alzheimer's disease, Parkinson's disease, ALS and many others. As revealing the existence of a common disease pathway would create the unique possibility to develop the same or a similar therapeutic strategy for all these devastating illnesses.
We will approach the above questions by using a combination of genetic mouse models, patient-derived human induced pluripotent stem cells, molecular biology tools, imaging, optogenetics and electrophysiology to reveal:

- The relation between spreading and neuronal degeneration.
- The role neuronal connectivity plays in spreading.
- The cellular and molecular components borrowed by toxic proteins to transcellular (neuron-neuron, neuron-glia) propagate.

This is done in state-of-the art *in vitro* and *in vivo* experimental designs, including co-cultures of mouse organotypical brain slices with human stem cell-derived neurons to address disease specific questions in a human related context.

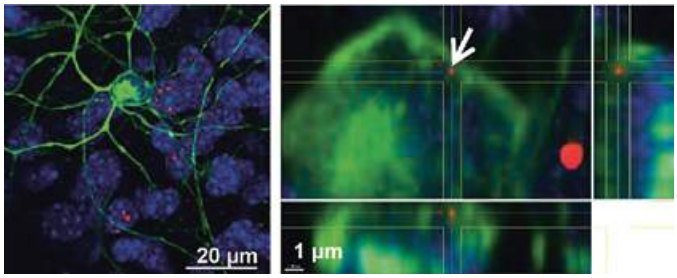

Fig. 1: Co-culture of organotypical brain slice obtained from Huntington's disease mouse (blue cells) with healthy human embryonic stem cell derived neurons (human neurons; green), reveals transcellular spreading of sick huntingtin protein to human neurons (red; right image, arrow).

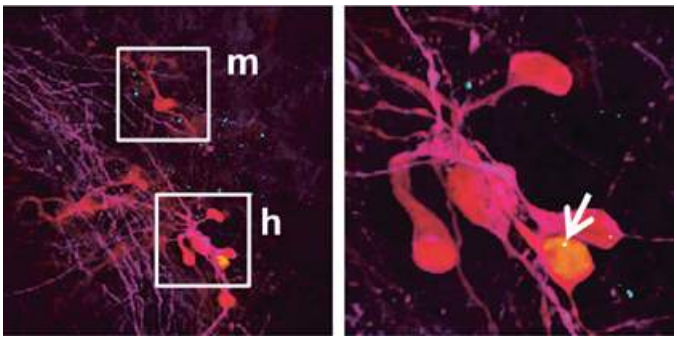

Fig. 2: Synaptic integration of human neurons in mouse neuronal network is here demonstrated with viral tracing technique, visualizing synaptic connectivity between healthy human neuron (h (yellow)) and Huntington's disease mouse neuron (m (red)). Right image shows the human neuron with sick huntingtin protein derived from the surrounding mouse cells (arrow).

Selected Publications

Pecho-Vrieseling E. (2016) Hunting cellular mechanisms underlying the spreading of misfolded protein pathology in the brain. Neuropathol Appl Neurobiol. 42(2):135–6

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*left during report period

Development and function of neuronal circuits in the central auditory system

Sounds and hearing play a pivotal role in human communication. People who suffer from central auditory processing abnormalities are affected in their daily lives and might not be able to appreciate even the most basic verbal communication. Tinnitus, in which phantom sounds are experienced in the absence of acoustic stimulation, is an example of pathology of the central auditory system. Ten percent of the human population suffers from auditory cortex disorders, yet we understand very little about its role in making sense of sounds.

In our lab we study the development and function of the auditory cortex. We combine optogenetics, *in vivo* physiology, voltage-sensitive dye imaging and behavioral assays to explore the role of its neuronal circuits. The goal of our research is to give a new insight into the function of the auditory system and to lead to new ways of reinstating normal connectivity in cases of abnormal signal processing.
Ongoing work is directed towards three main questions: 1) How do auditory cortical responses develop and how can they be modified? 2) What neuronal circuits are involved in specific sound features, and how do they influence behavior? 3) What influences does the environment have on regulating these auditory neuronal circuits?

Neuronal responses to different sound features develop asynchronously
Using *in vivo* electrophysiological recording and voltage-sensitive dye imaging in the mouse auditory cortex, we are characterizing the development of auditory responses to sounds of increasing complexity, like pure frequency tones, frequency- or amplitude-modulated sweeps. We also determine the time windows – known as critical periods for plasticity – during which brain organisation can be modified by passive sound exposure.
Our results indicate that responses to sound features of increasing complexity mature asynchronously. Passive exposure to these sound features changes neuronal circuit organisation during distinct time windows (Fig. 1). Interestingly, these critical periods coincide with the maturation of sound feature representation. This indicates that sensory development and plasticity involve the same cortical substrate. We are currently studying the underlying cortical circuits to shed a new light on how the brain processes different sounds during development.

Interneurons enhance tone frequency selectivity in auditory cortex
Using optogenetics combined with electrophysiology, we are studying the role of neuronal subpopulations in shaping responses to specific sound features. We are also assessing the behavioural consequences of controlling these neuronal subgroups in auditory relevant tasks. The subpopulations we now focus on are inhibitory neuron subclasses, like the parvalbumin-, somatostatin- or vasointestinal protein-expressing neurons.
Our results indicate that inhibitory neurons enhance tone frequency selectivity in response to pure frequency tones (Fig. 2). These changes are correlated with changes at the behavioural level: increasing frequency selectivity at the neuronal level with optogenetics also increases the ability to discriminate to sounds in a go/nogo behavioural task. We are currently determining whether different types of interneurons play a different role in this frequency selectivity, and also whether these subclasses of neurons have a different role in responding to more complex sounds.

Noise exposure modifies brain organisation differently in a developing than in a fully mature brain

By exposing mice to continuous white noise during several days, we are probing the influence of the environment on the rules regulating the organisation and plasticity of auditory circuits.

Our results indicate that the external environment can have very different consequences on a developing or a mature brain. We are currently determining the specific cell types and circuits modified by these environmental exposures, and to what extend these changes can be reversed. This research will have an impact on the way we look at the constant occupational noise that we and our children are exposed to on a daily basis, ranging from background music, construction work or even psychosocial stress.

Selected Publications

Favre MR, Barkat TR, La Mendola D, Khazen G, Markram H, Markram K. (2013) General developmental health in the VPA-rat model of autism. Front. Behav. Neurosci. 7,88

Barkat TR, Polley DB, Hensch TK. (2011) A critical period for auditory thalamocortical connectivity. Nature Neuroscience, 14(9), 1189–1194

Hackett TA*, Barkat TR*, O'Brien BJ, Hensch TK, Polley DB. (2011). Linking topography to tonotopy in the mouse auditory thalamocortical circuit. J. Neuroscience, 31(8), 2983–2995

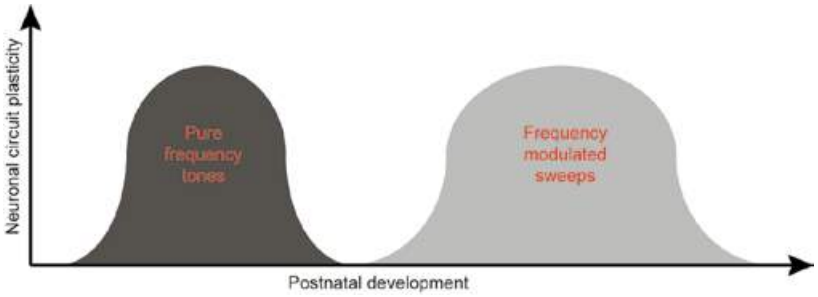

Fig. 1: Critical periods for plasticity are numerous and asynchronous.
The time window of enhanced plasticity to passive pure frequency tones exposure arises earlier than the one for frequency modulated sweep exposure.

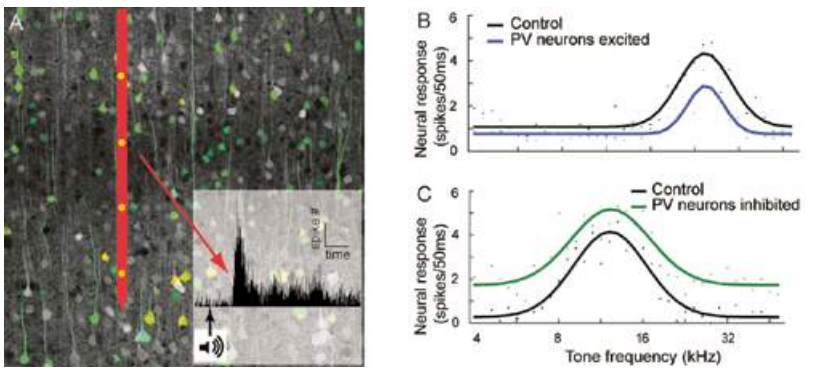

Fig. 2: Inhibitory neurons enhance tone frequency selectivity in auditory cortex
A. Schematic of electrophysiological recording in mouse auditory cortex. The red line illustrates an electrode shaft with 4 recording sites in different layers of the cortex. Inset: representative peristimulus time histogram following a 50ms pure frequency tone exposure. **B. C.** Example neural responses to pure frequency tones indicate that neurons respond stronger to some frequencies than to others. These frequency selectivities are enhanced (B) or decreased (C) when optogenetic manipulations activate or silence parvalbumin-expressing (PV) neurons, respectively.

Neurobiology

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Molecular Mechanisms of Myelin Formation and Maintenance in Health and Disease

Oligodendrocytes are the myelinating cells of the central nervous system. Their main function is to insulate axons to enable saltatory conduction. They further support neurons by providing continuous metabolic and trophic support. To fulfill these tasks oligodendrocytes maintain up to 50 single myelin sheaths supporting many axons at a time.

Oligodendrocytes derive from the neuroectoderm and are the last neural cells differentiating during development. Oligodendrocyte precursor cells (OPCs) proliferate, migrate and finally contact axons which they ultimately myelinate. This process is tightly controlled not only by a complex intrinsic oligodendrocyte differentiation program, but also by external reciprocal signaling processes such as the degree of neuronal differentiation. Due to their complex architecture and high metabolic demands, oligodendrocyte function can be easily disturbed; because of that they are often referred to as **"the most vulnerable cells of the CNS".** This is especially unfortunate in the case of disturbances of brain homeostasis. Many events can lead to such disturbances; among them inflammatory pathomechanisms of the CNS such as in **Multiple Sclerosis (MS).** In the context of inflammation, oligodendrocytes are generally believed to be simply victims of the inflammatory reaction, being lucky they survive. However, this view is probably too simple, and an increasing body of evidence demonstrates that oligodendrocytes can react and/or act if challenged by immune reactions (Fig. 1). They express various cytokines and chemokines, antigen presenting molecules and co-stimulatory molecules, complement and complement receptor molecules, complement regulatory molecules, tetraspanins, neuroimmune regulatory proteins as well as extracellular matrix proteins and many others. Their potential immunomodulatory properties can, at specific times and locations, influence ongoing immune processes as shown by numerous publications. Therefore, oligodendrocytes are well capable of immunomodulation, especially during the initiation or resolution of immune processes in which subtle signaling might tip the scale. A better understanding of the immunomodulatory oligodendrocyte can help to invent new, innovative therapeutic interventions in various diseases such as Multiple Sclerosis. **These potential immunomodulatory features of oligodendrocytes** are the topic of a recent review (Zeis *et al.*, Brain Research 2016).

Emerging as an important correlate of **neurological dysfunction in MS, extended focal and diffuse gray matter abnormalities** have been found and linked to clinical manifestations such as seizures, fatigue and cognitive dysfunction. To investigate possible underlying mechanisms we analyzed the molecular alterations in histopathological normal appearing cortical gray matter (NAGM) in MS. By performing a differential gene expression analysis of NAGM of control and MS cases we identified reduced transcription of astrocyte specific genes involved in **the astrocyte-neuron lactate shuttle (ANLS) and the glutamate-glutamine cycle (GGC)** (Fig. 2). Additional quantitative immunohistochemical analysis demonstrating a CX43 loss in MS NAGM confirmed a crucial involvement of astrocytes and emphasizes their importance in MS pathogenesis. Concurrently, a Toll-like/IL-1β signaling expression signature was detected in MS NAGM, indicating that immune-related signaling might be responsible for the downregulation of ANLS and GGC gene expression in MS NAGM. Indeed, challenging astrocytes with immune stimuli such as IL-1b and LPS reduced their ANLS and GGC gene expression *in vitro*. The detected upregulation of IL1B in MS NAGM suggests inflammasome priming. For this reason, astrocyte cultures were treated with ATP and ATP/LPS as for inflammasome activation. This treatment led to a reduction of ANLS and GGC gene expression in a comparable manner. To investigate potential sources

for ANLS and GGC downregulation in MS NAGM, we first performed an adjuvant-driven stimulation of the peripheral immune system in C57Bl/6 mice *in vivo*. This led to similar gene expression changes in spinal cord demonstrating that peripheral immune signals might be one source for astrocytic gene expression changes in the brain. IL1B upregulation in MS NAGM itself points to a possible endogenous signaling process leading to ANLS and GGC downregulation. This is supported by our findings that, among others, MS NAGM astrocytes express inflammasome components and that astrocytes are capable to release Il-1β *in vitro.* Altogether, our data suggests that immune signaling of immune- and/or central nervous system origin drives alterations in astrocytic ANLS and GGC gene regulation in the MS NAGM. Such a mechanism might underlie cortical brain dysfunctions frequently encountered in MS patients (Zeis *et al.*, BBI 2015).

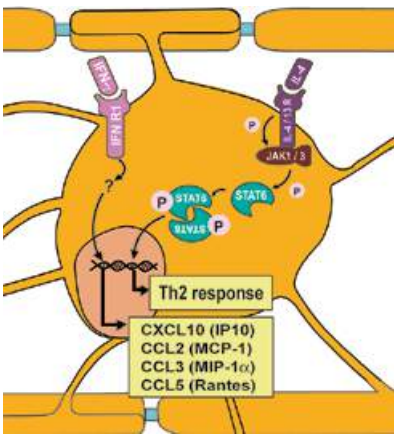

Fig. 1: Immune response-mediating pathways in oligodendrocytes. Analysis of proteins expressed by oligodendrocytes revealed that oligodendrocytes are able to express immune mechanisms-related proteins. Members of the STAT6 signaling pathway, such as IL-4R, IL13R, JAK1 and STAT6, were shown to be expressed by oligodendrocytes. This might indicate an anti-inflammatory "Th-2"-like response by oligodendrocytes. Furthermore, treatment of oligodendrocytes with a sub-lethal dose of IFN-γ and TNF-α led to the secretion of chemokines such as CXCL10 (IP-10), CCL2 (MCP-1), CCL3 (MIP-1a) and CCL5 (Rantes). Altogether, this indicates that oligodendrocytes might play an immune-modulating role MS (Zeis and Schaeren-Wiemers, 2008).

Selected Publications

Weil MT, Möbius W, Winkler A, Ruhwedel T, Wrzos C, Romanelli E, Bennett JL, Enz L, Goebels N, Nave KA, Kerschensteiner M, Schaeren-Wiemers N, Stadelmann C, Simons M. Loss of Myelin Basic Protein Function Triggers Myelin Breakdown in Models of Demyelinating Diseases. Cell Rep. 2016 Jul 12;16(2):314-22. doi:10.1016/j.celrep.2016.06.008. Epub 2016 Jun 23. PubMed PMID: 27346352; PubMed Central PMCID: PMC4949381

Zeis T, Enz L, Schaeren-Wiemers N. (2016) The immunomodulatory oligodendrocyte. Brain. Res. 2016 Jun 15; 1641(Pt A):139–48. doi: 10.1016/j.brainres.2015.09.021. Epub 2015 Sep 28. Review. PubMed PMID: 26423932

Zeis T, Allaman I, Gentner M, Schroder K, Tschopp J, Magistretti PJ, Schaeren-Wiemers N. (2015) Metabolic gene expression changes in astrocytes in Multiple Sclerosis cerebral cortex are indicative of immune-me- diated signaling. Brain Behav Immun. 48:313–25. doi: 10.1016/j.bbi.2015.04.013

Schmid D, Zeis T, Sobrio M, Schaeren-Wiemers N. (2014). MAL overexpression leads to disturbed expression of genes that influence cytoskeletal organization and differentiation of Schwann cells. ASN Neuro 6: 1759091414548916, first published on September 10, 2014 doi:10.1177/1759091414548916

Schmid D, Zeis T, Schaeren-Wiemers N. (2014) Transcriptional regulation induced by cAMP elevation in mouse Schwann cells. ASN NEURO 6(3):art:e00142. doi:10.1042/ AN20130031

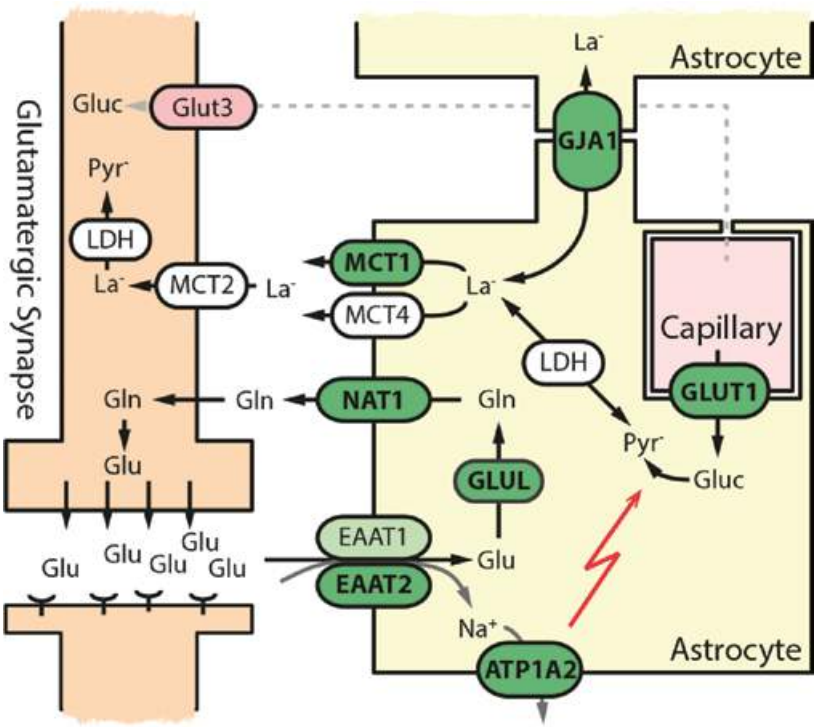

Fig. 2: Differential gene expression in normal appearing cortical gray matter of chronic MS. Schematic drawing of genes belonging to the ANLS and GGC. This model includes the following sequence of molecular events: Following increased synaptic activity glutamatergic neurons release the neurotransmitter glutamate into the synaptic cleft. Glutamate is avidly taken up by the astrocytes surrounding the synaptic cleft, via specific glial glutamate transporters (EAAT1 and EAAT2). EAATs co-transport glutamate with sodium ions increasing intracellular sodium concentration in the astrocyte and activating the energy dependent Na+/K+ ATPase pump (through the recruitment of the alpha 2 subunit). The corresponding hydrolysis of ATP leads to activation of astrocytic glycolysis, i.e. the degradation of glucose to pyruvate, which is then converted to lactate via lactate dehydrogenase (LDH). Lactate is then released via astrocytic monocarboxylate transporters (MCT1 and 4) into the extracellular space and from there taken up by the neurons (via MCT2). In neurons it serves as an energy substrate following its intracellular conversion to pyruvate by LDH. Genes which were found by qRT-PCR to be highly significantly ($p \leq 0.001$) downregulated in MS NAGM are shown in dark green (in bold), genes with a higher p-value showing a tendency to be downregulated are shown in light green. Genes showing a tendency to be upregulated are shown in light red. Red bolt indicates coupling of glutamate transport with glucose utilization. Abbreviations: Gluc = Glucose, Pyr-= Pyruvate, La- = Lactate, Glu = Glutamate, Gln = Glutamine.

Neuromuscular Research

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Translational Research in Neuromuscular Diseases

Our Neuromuscular Research Laboratory, Clinic of Neurology and Department of Biomedicine, focuses on the elucidation of pathophysiological mechanisms involved in neuromuscular diseases and on the development of therapeutic strategies. Myotonic dystrophy type I (DM1) is a disabling neuromuscular disease with no causal treatment available. It is the most prevalent muscular dystrophy in adults, affecting about 1 in 10'000 individuals. This disease is caused by expanded CTG trinucleotide repeats in the 3' UTR of the dystrophia myotonica-protein kinase gene *(DMPK).* On the RNA levels, expanded (CUG)n repeats form hairpin structures that sequester splicing-factors, such as muscleblind-like 1 (MBNL1). Lack of available MBNL1 leads to mis-regulated alternative splicing of many target pre-mRNAs, causing multisystemic involvement in DM1.

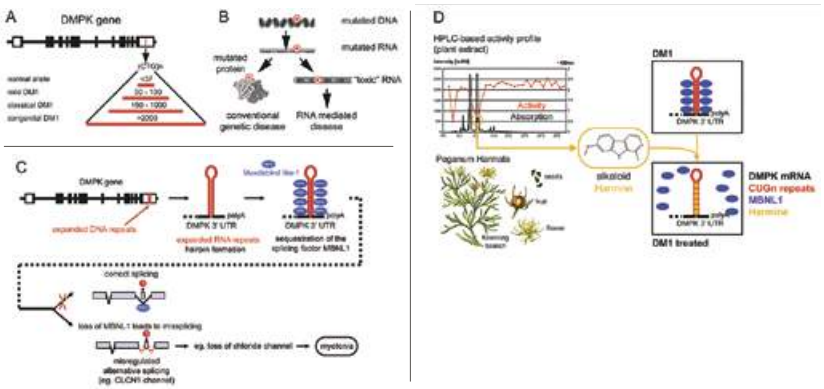

Fig. 1: The molecular basis of DM1 is an expansion of an unstable repeat sequence in the noncoding part of the DMPK gene (A). Severity of disease is correlated with the size of the repeat expansion (A). In DM1, the mutation is located in a noncoding region and does not alter the protein sequence, but leads to toxic RNA (B). The sequestration of the alternative splicing factor MBNL1 by toxic RNA leads to altered splicing of target pre-mRNAs like *CLCN1,* encoding muscle-specific chloride channel (ClC-1). This mis-splicing leads to ClC-1 deficiency and to myotonia (C). HPLC-based activity profiling identifies the alkaloid harmine as the active constituent in an extract from the roots of Peganum harmala capable of inhibiting the complex formation between expanded CUG repeat RNA and the splicing factor MBNL1 (D). (plant image from *www.drugs-forum.com;* cartoons adapted from *Herrendorff et al., JBC 2016; 291(33)17165–77* and *Kinter and Sinnreich, Swiss Med Wkly. 2014;144:w13916)*

In an effort to identify small molecules that liberate sequestered MBNL1 from (CUG)n RNA, we focused in a collaborative effort with the research group of Prof. Matthias Hamburger, Pharmazentrum Basel, specifically on small molecules of natural origin. We developed a DM1 pathomechanism-based biochemical assay and screened a collection of isolated natural compounds, as well as a library of over 2100 extracts from plants and fungal strains. HPLC-based activity profiling in combination with spectroscopic methods were used to identify the active principles in the extracts. Bioactivity of the identified compounds was tested in a human cell model and in a mouse model of DM1. We identified several alkaloids, including the beta-carboline harmine and the isoquinoline berberine, which ameliorated certain aspects of the DM1 pathology in these models. These compounds may provide pharmacophores for further medicinal chemistry optimization.

To investigate whether deregulation of central metabolic pathways such as AMPK/mTOR may also be implicated in the pathogenesis of DM1, we are currently examining human skeletal muscle biopsies, as well as human cell lines and DM1 mice challenged by different conditions. In parallel, we are analyzing whether DM1 mus-

cle shows alterations in the autophagy flux and/or proteasome activity by biochemical and histological means. Lastly, we are testing the consequences of the modulation of these metabolic pathways on myotonia by *ex-vivo* electrophysiological methods. We recently found that AICAR, an agonist of AMPK, markedly reduces myotonia in DM1 mice; the underlying mechanisms are currently being investigated.

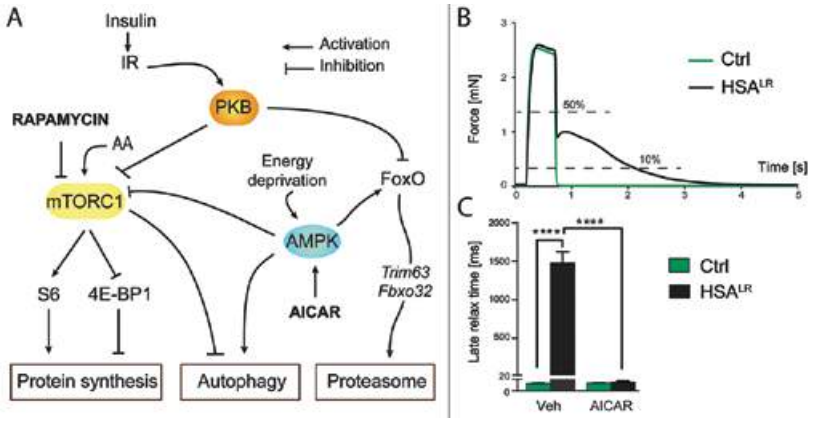

Fig. 2: A- Overview of the signaling pathways involved in proteostasis in muscle. B, C- *Ex vivo* measurement of the late relaxation time of EDL muscle reveals myotonia in HSA^{LR} mice. Figures adapted from *Brockhoff et al. J Clin Invest. 2017 Feb 1;127(2):549–563.*

In a broader context we are interested in the disruption of the proteostasis network as a possible pathomechanism for diseases affecting skeletal muscle. In collaboration with the research group of Prof. Markus Rüegg, Biozentrum Basel, we are investigating the implications of mTOR deregulation on muscle homeostasis and are studying the function and regulation of atrogenes, in particular the regulation of the F-box protein Fbxo32, which represents a potential therapeutic target against muscle wasting diseases.

Dysferlin is a transmembrane protein involved in surface membrane repair of muscle cells. Mutations in dysferlin cause the muscular dystrophies Miyoshi Myopathy and Limb Girdle Muscular Dystrophy Type 2B. In the laboratory we aim at developing novel treatment strategies for these diseases. Mouse models are valuable tools to test novel therapeutic approaches. A prerequisite for successful animal studies using genetic mouse models is an accurate genotyping protocol. Unfortunately, the lack of robustness of the currently available genotyping protocols for the Dysftm1Kcam mouse, a widely used dysferlin knock-out mouse model, has prevented efficient colony management. Initial attempts to improve the genotyping protocol based on the published genomic structure failed. These difficulties led us to analyze the targeted locus of the dysferlin gene of the Dysftm1Kcam mouse in greater detail. We found that instead of a deletion, the dysferlin locus in the Dysftm1Kcam mice carried a targeted insertion. This genetic characterization enabled us to establish a reliable method for genotyping of the Dysftm1Kcam mouse, and thus has made efficient colony management possible. These results will help the scientific community to use the Dysftm1Kcam mouse model for future studies on dysferlinopathies.

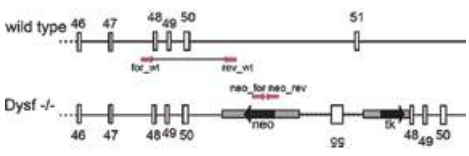

Fig. 3: Genetic characterization and improved genotyping of the dysferlin-deficient mouse strain Dysf tm1Kcam. Instead of a deletion, the dysferlin locus in the Dysf tm1Kcam mouse carries a targeted insertion. This genetic characterization enabled us to establish a reliable method for genotyping of the Dysf tm1Kcam mouse (red primers). (adapted from *Wiktorowicz et al., Skeletal Muscle2015, 5:32*)

Connection to Clinical Practice

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Interdisciplinary Neuromuscular Clinic

At our interdisciplinary Neuromuscular Clinic at the Department of Neurology we care for patients affected by a broad range of neuromuscular diseases. In collaboration with our colleagues from pathology, genetics, plastic surgery, pulmonary medicine, rehabilitation, ergo-, physio- and speech therapy as well as social services, we provide clinical and electrophysiological evaluation, perform muscle and nerve biopsies with histopathological and biochemical workup, genetic workup and counseling, rehabilitation, ergo-/physio- and speech therapy as well as assistance in social matters. Novel clinical observations are being worked up scientifically and form the basis for translational research projects.

Selected Publications

Brockhoff M, Rion N, Chojnowska K, Wiktorowicz T, Eickhorst C, Erne B, Frank S, Angelini C, Furling D, Rüegg MA, Sinnreich M, Castets P. Targeting deregulated AMPK/mTORC1 pathways improves muscle function in myotonic dystrophy type I. J Clin Invest. 2017 Feb 1;127(2):549–563. doi: 10.1172/JCI89616. Epub 2017 Jan 9

Pröbstel AK, Schaller A, Lieb J, Hench J, Frank S, Fuhr P, Kappos L, Sinnreich M. Mitochondrial cytopathy with common MELAS mutation presenting as multiple system atrophy mimic. Neurol Genet. 2016 Nov 17;2(6):e121. eCollection 2016 Dec.

Herrendorff R, Faleschini MT, Stiefvater A, Erne B, Wiktorowicz T, Kern F, Hamburger M, Potterat O, Kinter J, Sinnreich M. Identification of Plant-derived Alkaloids with Therapeutic Potential for Myotonic Dystrophy Type I. J Biol Chem. 2016 Jun 13. pii: jbc.M115.710616

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Perioperative Patient Safety

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Skeletal muscle calcium homeostasis under normal and pathological conditions

Calcium is a universal second messenger regulating different biological functions from muscle contraction, to gene transcription and cell death. In skeletal muscle, Ca2+ regulates contraction and relaxation and alterations in its intracellular concentration can lead to neuromuscular disorders. Investigations carried out during the past decade have shown that in more than 50 % of the cases, Central Core Disease, Multiminicore disease and Malignant Hyperthermia are linked to point mutations in the gene encoding the skeletal muscle sarcoplasmic reticulum calcium release channel ryanodine receptor (RyR1).

There are three isoforms of RyR that are expressed in different tissues; type 1 is preferentially expressed in skeletal muscles but recent data has shown that it is also expressed in some areas of the central nervous system, in some immune cells and in smooth muscle cells. These results imply that mutations in RYR1 may lead to alterations of Ca2+ homeostasis not only in skeletal muscle, but also in other tissues expressing this intracellular calcium release channel. Indeed ryanodinopathies have recently been implicated in other clinical conditions such as bleeding disorders, sepsis and intensive care polyneuropathiy, broadening the clinical spectrum of disorders linked to altered RyR1functions. Interestingly, type 3 RyR which was reported to be expressed at low levels in many tissues, appears to be the predominant isoform in extraocular muscles and we are currently investigating its role in this group of muscles, by using a RYR3 KO animal mouse model.

Our research also focuses on different aspects of calcium regulation in skeletal muscle under normal and pathological conditions and on the identification of pathomechanisms in congenital muscle disorders. While most dominant RYR1 mutations affect Ca2+ homeostasis by changing the biophysical properties of the RyR1 Ca2+ channel, the mode of action of recessive RYR1 mutations is more elusive, especially since at the level of myotubes, effects on Ca2+homeostasis are not prominent. On the other hand, striking changes occur in the patient's muscles. Such changes include a drastic decrease of RyR1 protein content, depletion of muscle specific miR-1 and 1-133, as well as depletion of miR-22 and -124 that specifically bund to the 3'UTR of the human RYR1, hyper-methylation of RYR1 CpG island and increased content of HDAC-4 and HDAC-5.

In order to gain insight into the mechanisms causing the epigenetic modifications and validate whether they represent valid pharmaceutical targets we have generated two mouse models harboring RYR mutations, using the CRISPR/Cas9 gene editing technology. The mutations we chose were originally identified in a severely affected patient with Multiminicore disease who harbored a premature stop codon in exon 36 (RyRGln1970X) and a missense mutation in exon 91 (RyRAla4329Asp). Ex-

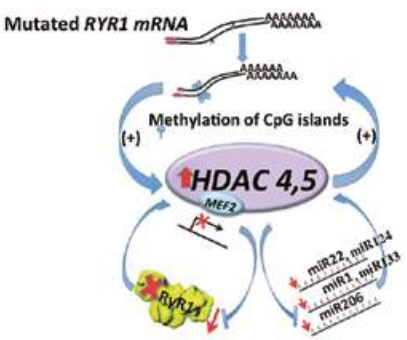

Fig. 1: Cartoon depicting how mutations in *RYR1* lead to a decrease in RyR1 content thereby leading to weak muscles. Mutations lead to DNA hyper-methylation and HDAC-4/HDAC-5 over-expression. This causes mef2 sequestration thereby inhibiting transcription of genes regulated by mef2, including the *RYR1* and muscle-specific miRs. A decrease in RyR1 would severely affect muscle excitation-contraction coupling since this calcium channel is a central player in this mechanism, releasing the calcium necessary for muscle contraction from the sarcoplasmic reticulum.

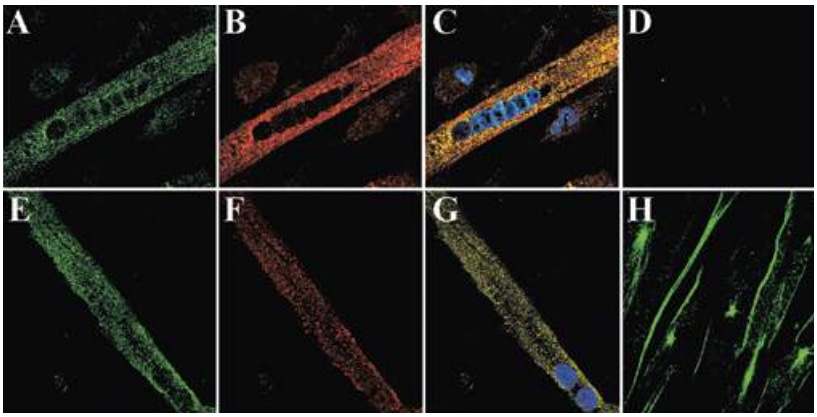

Fig. 2: Cellular distribution of RyR1 and $Ca_v1.1$ in differentiated *orbicularis oculi*-derived myotubes. Human myotubes were visualized with a Nikon A1R confocal microscope equipped with a CFI Apo TIRF 100X objective (1.49 N.A.) and stained as described in the Materials and Methods section. Top panels *orbicularis oculi,* bottom panels EOM. Panels **A** and **E** anti-$Ca_v1.1$ (green), **B** and **F** anti-RyR1 (red), **C** and **G,** merged image of anti-RyR1, anti-$Ca_v1.1$ and DAPI (blue); orange pixels show co-distribution of RyR1 and $Ca_v1.1$. Panels D and H, anti-$Ca_v1.2$ (green). Bar indicates 20 µm

tensive biochemical, physiological and cellular characterization of the mice models carrying single heterozygous and compound heterozygous mutations will help identify biomarkers that could be used to monitor disease progression or regression in patients. Of importance, similar epigenetic modifications may occur in the muscles of patients affected by other congenital myopathies (nemaline, myotubular and SEPN1-related myopathies). Thus discovering a common pharmacological target downstream the primary genetic defect could potentially benefit a large number of patients.

Other important areas of research focus on the role of calcium influx in skeletal muscle excitation contraction coupling as well as characterizing the role of SRP-35, a 35 kDa retinol dehydrogenase present in skeletal muscle sarcoplasmic reticulum. For the latter experiments we have created a transgenic mouse model over-expressing SRP35 in skeletal muscle. Such mice show increased exercise performance and increased glucose metabolism. We think that this model will offer important insight into the identification of molecular components coupling muscle activity to metabolism and may help identify potential molecular targets for the treatment of age-associated dismetabolic disorders such as type 2 diabetes.

Selected Publications

Lopez RJ, Byrne S, Vukcevic M, Sekulic-Jablanovic M, Xu L, Brink M, Alamelu J, Voermans N, Snoeck M, Clement E, Muntoni F, Zhou H, Radunovic A, Mohammed S, Wraige E, Zorzato F, Treves S, Jungbluth H. (2016) A RYR1 mutation associated with Malignant Hyperthermia is also associated with bleeding abnormalities. Science Signal. 9:435 ra68

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Sekulic-Jablanovic M, Ullrich ND, Goldblum D, Palmowski-Wolfe A, Zorzato F, Treves S. (2016) Functional characterization of orbicularis oculi and extraocular muscles. J. Gen. Physiol. 147:395–406

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Vukcevic M, Zorzato F, Keck S, Tsakiris DA, Keiser J, Maizels RM, Treves S. (2013) Gain of function of the immune system caused by a ryanodine receptor 1 mutation. J. Cell Sci. 126:3485–3492

Connection to Clinical Practice

Anesthesiology – a physiology lab?

Once feared as the most dangerous part of surgery, modern anesthesia has become very safe with an anesthesia-related mortality of below 1 in 200'000. Monitoring under general anesthesia includes heart rate, blood pressure, cardiac output, resistance, oxygen consumption, CO^2 production, minute ventilation, acid base status, neuromuscular function, urine output and much more. Today anesthesia has an impressive safety record. Nevertheless some diseases such as malignant hyperthermia (MH) are still life threatening. MH is a classical pharmacogenetic disease, triggered by anesthetic agents and characterized by a hypermetabolic state. Research has identified causative mutations in RYR1 in many susceptible individuals. However the genetic identity of some susceptible individuals is still unknown and needs to be determined in order to induce safe anesthesia. In addition neuromuscular blocking agents and volatile anesthetics can trigger severe skeletal muscle damage (myolysis, rhabdomyolysis) and hyperkalemia in patients with neuromuscular diseases. Many open questions on muscle physiology and calcium homeostasis are still to be understood in order identify patients at risk. Research on skeletal muscle, excitation-contraction coupling and calcium homeostasis has the potential to further increase perioperative patient safety.

Stem Cells and Regenerative Medicine

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Stem cell research and regenerative medicine are major pillars within the Department of Biomedicine (DBM) and the life science strategy of the University of Basel. The last decade has seen substantial progress in identifying and isolating stem cells from different adult tissues and embryonic origin, which can be induced to differentiate into various specific cell-types relevant to regenerative medicine. The groups of this focal area are active in various aspects of this fascinating field with relevance to basic, mechanistic and clinically applied, translational research.

The basic research efforts aim to identify and isolate stem cells and understand how stem cells are maintained in their normal niches within the embryo and/or the body. As such, several groups are studying how stem cells of the blood are maintained in the bone marrow, differentiate into the various different cell-types of the hematopoietic system, and how their differentiation potential is altered in malignant states that are caused by aberrant stem cell-based cancers (e.g. leukemia or lymphomas). The close interactions of clinical with basic researchers allow bridging the gap between fundamental and translational research. For example, attempts to grow and differentiate mesenchymal stem cells from human and mouse bone marrow *in vitro* into different cell- and tissue-types, aim at developing cartilage and bone replacement therapies that can be translated to the clinic. The knowledge gained from these studies forms the basis for designing and developing clinically applicable tissue engineering strategies and in moving toward regenerative medicine.

One of the major aims of regenerative medicine is to reactivate and support the regenerative potential of the body in a controlled manner. To this aim, understanding the normal regulation of organogenesis and tissue homeostasis is crucial. While first attempts have given encouraging results, it is important to gain a much better knowledge of how stem cells interact with their niche to maintain their multi-potency and give rise to daughter cells that undergo transient amplification upon leaving the niche. These populations of transient amplifying cells will then initiate their specification and differentiation in a controlled manner. Our challenge is to establish culture conditions where stem cells can be maintained and their specification and differentiation into functional tissues can be induced in an efficient and precisely controlled manner. Any functional organ and tissue will consist of well-organized and functionally interacting cells with different identities. Therefore, it is important to e.g. understand the role of embryonic signaling centers in tissue patterning/organization and cell-type specification/differentiation.

The knowledge gained from analyzing cell-type, tissue specification and organogenesis during normal embryonic development is highly relevant to directed engineering of tissues from progenitor and/or stem cells. So-called induced pluripotent stem (iPS) cells – adult cells (e.g. skin cells) reprogrammed into stem-like cells – are increasingly used as they can be relatively easily obtained from patients for cell differentiation and tissue engineering studies. The generation and analysis of iPS cells fits the strategy of the DBM to promote collaborative efforts between basic research groups and clinicians with the aim to significantly reduce the gap between bench and bedside. In addition to interactions within the DBM, there are numerous collaborations with groups at the Biozentrum, FMI and the D-BSSE, which

are funded by network grants such as Sinergia and SystemsX.ch. Many of our groups are actively participating in the Basel Stem Cell Network, which is one of the Competence Centers within the Life Sciences at the University of Basel. There, stem cell researchers have the opportunity to closely interact and collaborate with developmental biologists, geneticists and even mathematicians with the objective to foster interdisciplinary and innovative research.

Last but not least, with Verdon Taylor and Claudia Lengerke, we recently appointed two stem cell experts in the fields of brain development and hematopoietic stem cell signaling. Their groups help strengthening both basic and translational research efforts in this rapidly emerging and highly competitive research field.

Cell and Gene Therapy

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Therapeutic angiogenesis from vascular biology to regenerative medicine

Therapeutic angiogenesis aims at restoring blood flow to ischemic tissues by growing new vessels. Our research focuses on the basic principles governing vascular growth and their translation into rational approaches to: 1) treat ischemic diseases and 2) improve the vascularization of tissue-engineered grafts. We use precursor cells genetically modified to express controlled levels and combinations of factors, combining the specific advantages of cell and gene therapy.
Vascular endothelial growth factor (VEGF) is the master regulator of vascular growth. However, uncontrolled expression causes vascular tumors (angiomas). By the close interaction of basic scientists and clinical surgeons, we are developing novel methods to deliver the VEGF gene alone or in combination with modulating factors to increase its safety and efficacy *in vivo*, using transduced progenitors, gene therapy vectors and controlled release of recombinant proteins by smart biomaterials. Research is funded by Swiss agencies (SNF and Swiss Heart Foundation) and the European Union (FP7 and H2020).

1) Controlled VEGF delivery for therapeutic vascularization
We previously found that the transition between normal and aberrant angiogenesis depends on the VEGF amount in the microenvironment around each producing cell rather than on the total dose, since VEGF remains tightly localized in the extracellular matrix (Ozawa & Banfi 2004). In order to translate this biological concept into a clinically applicable approach, we developed a high-throughput FACS-based technology to rapidly purify transduced progenitors expressing specific VEGF levels (Misteli 2010). Controlled VEGF expression by FACS-purified progenitor populations could induce effective vascularization both inside and outside of thick, engineered cardiac patches (Marsano 2013; Boccardo & Gaudiello 2016), therapeutic angiogenesis in ischemic myocardium (Melly 2012 and manuscript submitted) and increased *in vivo* vascularization of osteogenic grafts (Helmrich 2013; Largo & Di Maggio, manuscript submitted).
To avoid the need for genetic modification and improve clinical applicability, in collaboration with Jeffrey Hubbell (EPFL, Lausanne) we developed a state-of-the-art biomaterial platform based on fibrin hydrogels that enables independent control of the dose and duration of release of matrix-bound growth factors, by which we could identify a 500-fold range of VEGF concentrations inducing only physiological capillary networks, which were long-term stable and therapeutically effective in ischemic wounds (Sacchi 2014).

2) Cellular and molecular mechanisms of VEGF dose-dependent angiogenesis
Our understanding of angiogenic mechanisms is mostly based on developmental models, in which new vessels sprout to vascularize tissues. However, we found that VEGF delivery to skeletal muscle, at the doses needed for functional benefit, induces angiogenesis without sprouting, but by circumferential enlargement of vessels, followed by longitudinal splitting ("intussusception", Gianni-Barrera 2013; Fig. 1). The molecular basis for the induction of sprouting or intussusception by VEGF is provided by opposite patterns of activation of Notch1 signaling (Gianni-Barrera, manuscript submitted). We also found that the transition between normal and aberrant angiogenesis is not an intrinsic property of VEGF dose, but depends on the balance between VEGF-induced endothelial stimulation and vascular maturation mediated by pericyte recruitment by PDGF-BB (Banfi 2012).
On the other hand, new vessels regress if VEGF delivery is shorter than about 4 weeks: we identified Semaphorin3A as a molecular target to accelerate the sta-

bilization and persistence of new vessels despite transient VEGF delivery (Groppa 2015; Fig. 2). Taking advantage of the highly controlled cell-based gene delivery platform we developed, we are currently pursuing a systematic investigation of the mechanisms that regulate the switch between normal and aberrant angiogenesis *in vivo*, through the analysis of the stage-specific mRNA and miRNA transcriptomes of ex-vivo purified vascular cells to identify novel and more specific molecular targets for therapeutic angiogenesis.

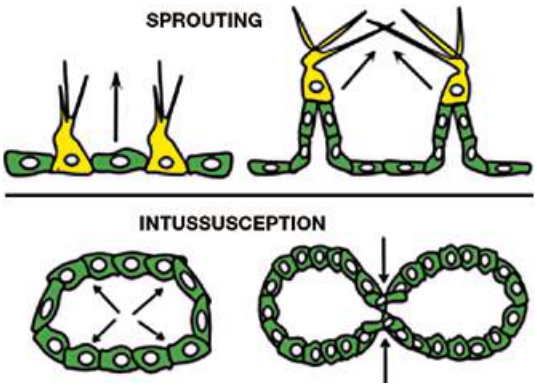

Fig. 1: The two alternative modes of vascular growth: migration into avascular tissue (sprouting) or circumferential enlargement and longitudinal splitting of pre-existing vessels (intussusception).

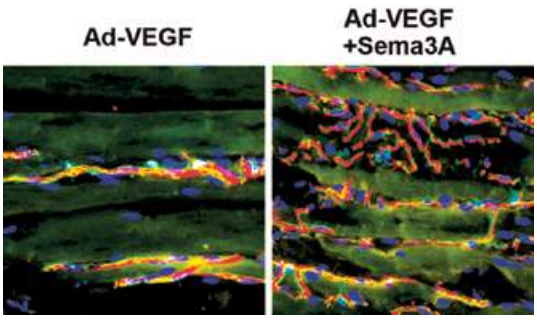

Fig. 2: Transient VEGF expression, e. g. by adenoviral vectors (Ad-VEGF), is clinically desirable to ensure safety, but it is insufficient to allow stabilization of induced vessels, which promptly regress upon cessation of the VEGF stimulus (left). Treatment with Semaphorin3A (Sema3A) accelerates the stabilization of newly induced vessels, preventing their regression despite transient VEGF delivery (right).

Selected Publications

Gianni-Barrera R, Burger M, Wolff T, Heberer M, Schaefer DJ, Gürke L, Mujagic E, Ban A. (2016) Long-term safety and stability of angiogenesis induced by balanced single-vector co-expression of PDGF-BB and VEGF164 in skeletal muscle. Sci Rep 6, 21546

Groppa E, Brkic S, Bovo E, Reginato S, Sacchi V, Di Maggio N, Muraro MG, Calabrese D, Heberer M, Gianni-Barrera R, *et al.* (2015) VEGF dose regulates vascular stabilization through Semaphorin3A and the Neuropilin-1+ monocyte/TGF- beta1 paracrine axis. EMBO Mol Med 7, 1366–1384

Martino MM, Brkic S, Bovo E, Burger M, Schäfer DJ, Wolff T, Gürke L, Briquez PS, Larsson HM, Gianni-Barrera R, *et al.* (2015) Extracellular matrix and growth factor engineering for controlled angiogenesis in regenerative medicine. Front Bioeng Biotechnol 3, 45 doi: 10.3389/ fbioe.2015.00045

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Gianni-Barrera R, Trani M, Fontanellaz C, Heberer M, Djonov V, Hlushchuk R, Ban A. (2013) VEGF over-expression in skeletal muscle induces angiogenesis by intussusception rather than sprouting. Angiogenesis 16, 123–136

Connection to Clinical Practice

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Prof. Dr. Dirk J. Schäfer,
Prof. Dr. Friedrich Eckstein

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Therapeutic angiogenesis and tissue regeneration

The goal of the group is to translate the basic biological principles controlling the physiological generation of normal and functional vascular networks into the design of rational strategies to induce therapeutic growth of new blood vessels. We are currently pursuing this concept in three main areas of clinical interest:

1) To induce controlled angiogenesis in the myocardium and generate vascularized cardiac patches with transduced and FACS-purified VEGF-expressing adipose tissue-derived mesenchymal progenitors, in order to improve contractile function in the ischemic heart (Dr. med. L. Melly and Prof. F. Heckstein, Cardiac Surgery USB).
2) To achieve rapid vascularization of the inner core of clinical-size osteogenic grafts in order to favor progenitor survival and differentiation, leading to improved bone formation, by using dose- and time-controlled delivery of fibrin-bound VEGF protein together with bone marrow-derived osteoprogenitors (Dr. med. M. Burger, Dr. med. A. Lunger and Prof. D. J. Schäfer, Plastic and Reconstructive Surgery USB).
3) To achieve therapeutic angiogenesis in chronically ischemic muscle tissue or associated non-healing wounds for the treatment of peripheral artery disease patients, by dose- and time-controlled delivery of recombinant angiogenic factors by smart biomaterials (Dr. med. E. Mujagic, PD Dr. med. T. Wolff and Prof. L. Gürke, Vascular Surgery USB).

Inner Ear Research

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Unravelling molecular mechanisms of auditory hair cell loss to find new therapeutic possibilities to treat hearing loss

Hearing loss has a huge impact on the affected individual as well as on our society. Not only is one baby out of 1000 born with hearing loss, but also more than 50% of people older than 65 years suffer from hearing loss. Hearing loss of adult onset is one of the ten leading causes of disability-adjusted life years globally. It is estimated that 278 million persons worldwide suffer from disabling hearing loss (two-thirds of whom reside in developing countries). The impact of hearing loss on health care costs will very likely increase in the future considering the envisaged improvement in life expectancy.

The degeneration of inner ear sensory cells located in the cochlea, known as hair cells underlies most forms of sensorineural hearing loss. Sensorineural hearing loss is difficult to treat, since it creates both loss of sensitivity and distortion. While hearing aids can increase sensitivity, they often do not overcome the distortion of sound caused by loss of hair cells. Therefore, in order to pave the road for new prophylactic and therapeutic approaches for sensorineural hearing loss, it is of the essence to thoroughly investigate molecular events involved in hair cell damage and death. Once we understand these molecular events we might try to block apoptosis signalling pathways while enhancing cell survival pathways, finally this will result in enhanced hair cell survival.

During the last couple of years, our research has been centered on the molecular mechanisms involved in hair cell damage and death. It has been demonstrated that signaling pathways exist that promote hair cell death and interestingly, it has also been shown that there are opposing pathways that promote hair cell survival. Different molecules have been found who influence these pathways, among them inhibitors who inhibit the JNK signaling pathway, apoptosis inhibitors, erythropoietin, somatostatin and octreotide which bind to the somatostatin receptor, and others. Also the phosphatidylinositol 3-kinase (pi3k) /Akt pathway has been studied in detail and its role in hair cell death and survival has been studied. Lately, we have founded a spin off company (Strekin AG, Basel) to explore pioglitazone and its effect on auditory hair cells. Currently, Strekin is running a clinical phase 2 study to investigate whether pioglitazone can protect residual hearing during cochlea implant surgery.

Selected Publications

Bertoli S, Bodmer D (2016). Effects of age and task difficulty on ERP responses to novel sounds presented during a speech-preception-in-noise test. Clin Neurophysiology 15:S1388–2457

Levano S, Bodmer D (2015). Loss of STAT1 protects hair cells from ototoxicity through modulation of STAT3, c-Jun, Akt, and autophagy factors. Cell Death & Disease, 6:e2019

Brand Y, Levano S, Radojevic V, Monge A, Setz C, Ryan AF, Pak K, Hemmnings B, Bodmer D (2015). All Akt isoforms are involved in normal hearing, but only Akt2 and Akt3 are involved in auditory hair cell survival in the mammalian inner ear. PlosOne 10(3):e0121599

Brand Y, Radojevic V, Sung M, Wei E, Setz C, Glutz A, Leitmeyer K, Bodmer D (2014). Role of somatostatin receptor-2 in gentamicin-induced auditory hair cell loss in the mammalian inner ear. PlosOne 9(9):108146

Radivojevic V, Bodmer D (2014). Expression and localization of somatostatin receptor type 3, 4 and 5 in the wild-type, SSTR1 and SSTR1/SSTR2 knockout mouse cochlea. Cell and tissue research 358(3): 717–27

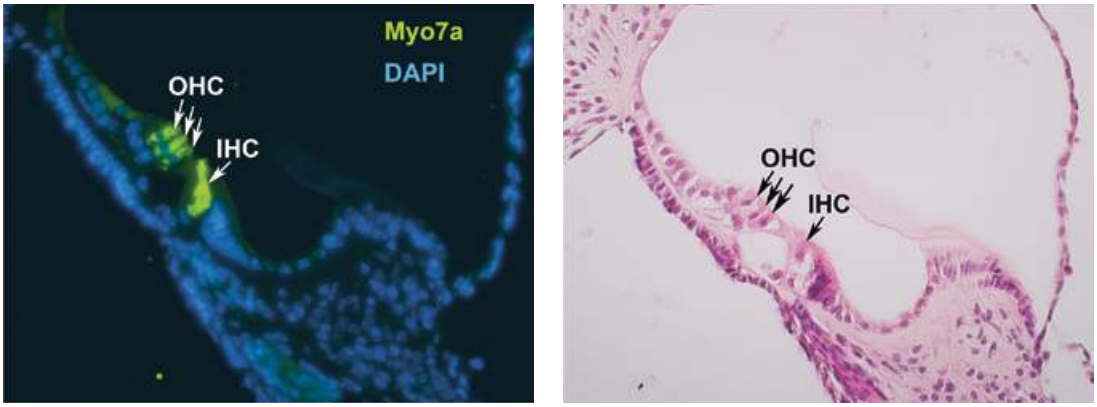

Fig. 1: Transverse sections of the cochlear duct from adult wild type mice. Upper panel highlights the auditory inner and outer hair cells stained with myosin 7a antibody (Myo7a, green). Lower panel shows haematoxylin-eosin staining of cochlear structures. OHC, outer hair cells, IHC, inner hair cells.

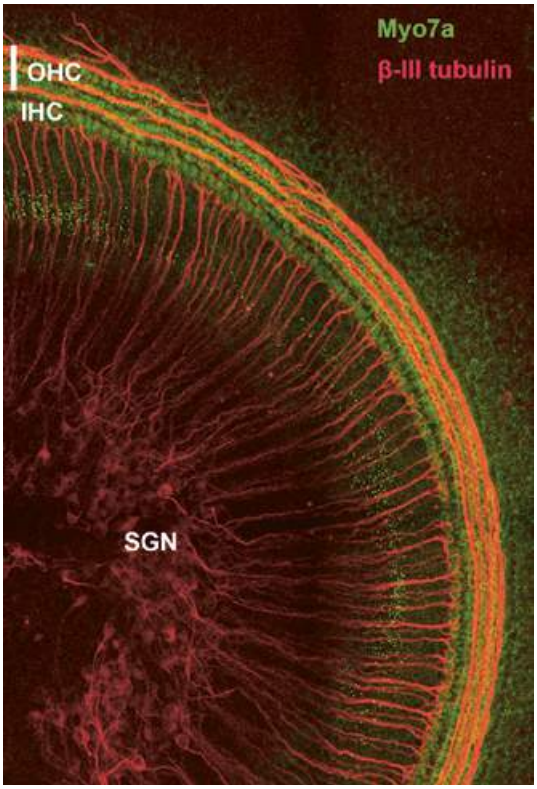

Fig. 2: Whole mount staining of the middle turn of the organ of Corti from P4 mice highlighting the tree outer hair cells (OHC) and the inner hair cells (IHC) stained with myosin 7a antibody (Myo7a, green); and spiral ganglion neurons (SGN; β-III tubulin, red).

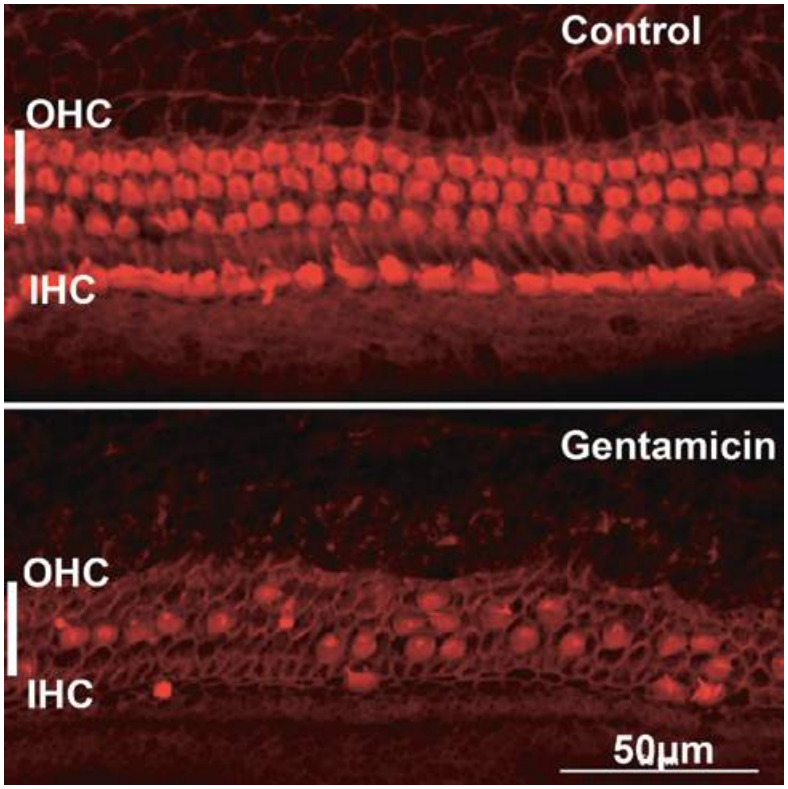

Fig. 3: Hair cell damage in the organ of Corti of neonatal mice induced by gentamicin. Phalloidin-stained hair cells from middle cochlear turn treated with and without gentamicin for 24 hours.

Cardiobiology

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*left during report period

Regulation of protein turnover and energy metabolism in cardiac disease

Heart failure is a syndrome in which the heart is unable to adequately perfuse the body's organs with blood. It adversely affects the wellbeing of patients as it may cause muscle weakness and atrophy, dyspnoe, as well as organ damage and dysfunction. To be able to function as a pump that provides oxygen and nutrients to our whole body, the heart itself consumes large amounts of energy. A tight regulation of the use of all available resources, including cellular proteins, becomes particularly critical in disease states where metabolism must increase to maintain cardiac performance. In fact, a fundamental mechanism that underlies heart failure is the failure to metabolically adapt. Our laboratory investigates cellular mechanisms and signaling pathways that regulate cardiac protein turnover and energy metabolism.

Functions of mTORC1 and mTORC2 in the heart

We analyzed the function of the intracellular metabolic regulator mammalian target of rapamycin (mTOR), which has distinct functions depending on whether it is part of mTOR complex (mTORC)1 or mTORC2. Using tissue-specific inducible knockout approaches, we have been able to show that mTORC1 is required for basal cardiac function and that it becomes even more important in physiological or pathological cardiac stress induced by voluntary wheel running or aortic constriction. In mTORC1-deficient mice, pathological pressure overload caused dilated cardiomyopathy without a prior phase of adaptive hypertrophy due to a lack of adaptive cardiomyocyte growth via blunted protein synthesis capacity, and associated with reduced mitochondrial content, a shift in metabolic substrate use and increased apoptosis and autophagy. In contrast, rictor-deficient hearts (rictor is a specific component of mTORC2) are normal during growth or adulthood under basal conditions. We found that pressure overload significantly increases rictor protein along with PKCβII and PKCδ phosphorylation in control mice, but not in cardiac rictor knockout mice. Pressure-overload causes hypertrophy with maintained function in controls, but leads to systolic dysfunction of rictor-deficient hearts without having any effects on cardiac weight, hypertrophy markers or fibrosis. These data suggest that mTORC2 regulates metabolism and contractility of the heart via PKCII and PKCδ (Fig. 1, Xu, 2015; Shende, 2016).

Cardio-protective mechanisms of neuregulin1β

Neuregulin1β (Nrg1β) has beneficial effects in a range of cardiac disease models and ongoing clinical trials are investigating its therapeutic value in heart failure. The mechanisms that underlie the cardio-protective actions of Nrg1β are poorly understood. We investigated whether Nrg1β modulates cardiomyocyte metabolism and whether mTOR is implicated in its cardio-protective effects. We found that Nrg1β stimulates glucose uptake in cardiac myocytes via ErbB2/ErbB4 heterodimers and by activating PI3Kα, Akt and AS160 in a similar manner as insulin and insulin-like growth factor-I (Fig. 2, Pentassuglia, 2016). In our ongoing studies, we are assessing to what extent IRS-1 is implicated and whether the identified mechanism can be exploited under pathological conditions *in vivo*.

Obesity and diastolic dysfunction

Approximately 50% of heart failure patients have a preserved ejection fraction, which means that the fraction of total blood present after completion of the filling phase that is pumped out of the heart into the circulation is maintained. HFpEF has been associated with impaired filling of the heart during diastole. As both the systolic and diastolic parts of the cardiac cycle depend on the availability of high

amounts of ATP, we are relating specific metabolic adaptations to contraction and relaxation efficiencies of the heart. To this end we established hypertension and diet-induced-obesity models that we follow using echocardiography prior to terminal invasive hemodynamic (pressure-volume loop) analysis, followed by molecular and microscopic analysis. We use both genders as well as ovariectomized mice for these studies while hoping to provide a fundamental basis for stratified strategies to prevent or treat cardiac dysfunction in both genders.

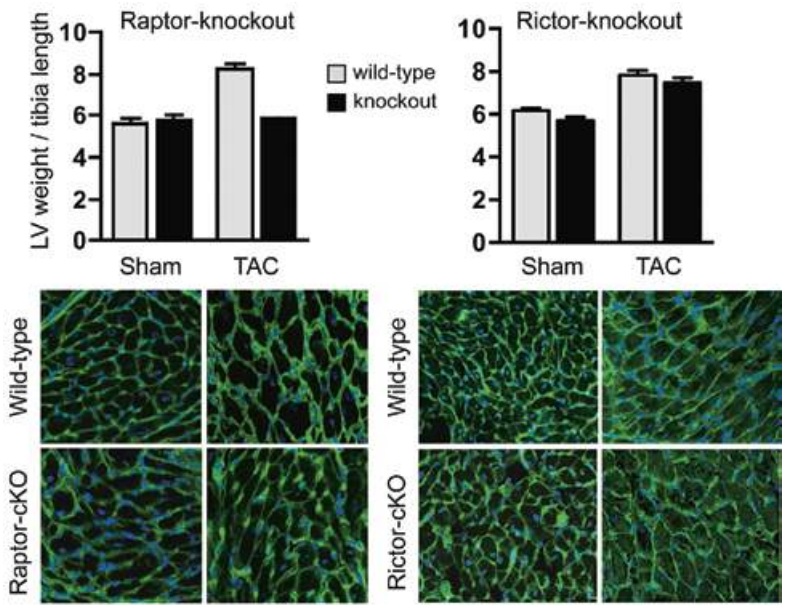

Fig. 1: Effects of cardiac raptor-deficiency (mTORC1 inactivation, left) and rictor-deficiency (mTORC2 inactivation) on left ventricular (LV) weight (top) and cardiomyocyte cross-sectional area (bottom, wheat germ agglutinin staining) in mice that were either sham-operated or exposed to transverse aortic constriction (TAC).

Selected Publications

Shende P, Xu L, Morandi C, Pentassuglia L, Heim P, Lebboukh S, Berthonneche C, Pedrazzini T, Kaufmann BA, Hall MN, *et al.* (2016) Cardiac mTOR complex 2 preserves ventricular function in pressure-overload hypertrophy. Cardiovasc Res 109, 103–114

Pentassuglia L, Heim P, Lebboukh S, Morandi C, Xu L, Brink, M. (2016) Neuregulin-1β promotes glucose uptake via PI3K/Akt in neonatal rat cardiomyocytes. Am J Physiol Endocrinol Metab 310, E782–794

Xu L, Brink M. (2016) mTOR, cardiomyocytes and inflammation in cardiac hypertrophy. Biochim Biophys Acta 1863. 1894–1903

Xu L, Shende P, Morandi C, Pentassuglia L, Heim P, Lebboukh S, Kaufmann BA, Berthonneche C, Pedrazzini T, Hall MN, *et al.* (2015) Regulators and effectors of mTORC2 in the heart. J Mol Cell Cardiol 86, S42–S43

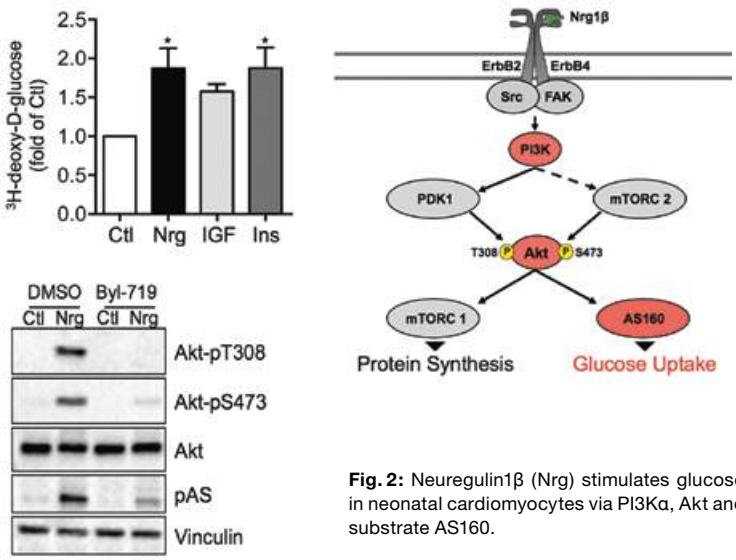

Fig. 2: Neuregulin1β (Nrg) stimulates glucose uptake in neonatal cardiomyocytes via PI3Kα, Akt and the Akt substrate AS160.

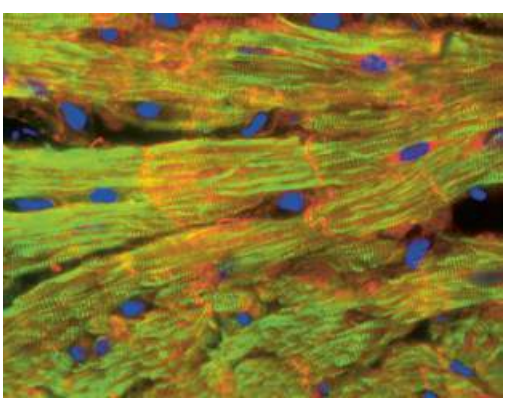

Fig. 3: Cardiac localization of the glucose transporter GLUT4 (red) and Troponin T (green). The nuclei are visualized with DAPI (blue).

Gynecological Endocrinology

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Ovarian and reproductive disease modelling using stem cell technology

We combine human stem cell technology to unravel the original of signaling pathways in reproductive disease and in early embryonic development. As a putative mediator of inhibin signaling in the ovary we originally identified an E3 ubiquitin ligase for inhibin B receptor, EULIR, which is now renamed as HECTD1. In order to study its function, we generated a new mutant mouse model using the gene trap strategy. Homozygous mutant mice resulted in a early embryonic lethality, displaying severe growth retardation, abnormal placental development and defective of neural tube closure (e. g. spina bifida). Hectd1 expression is detected in specific cell populations of multiple tissues (Fig. 1), is regulated by insulin and by heat and hypoxia. E3 ligase activity of Hectd1 regulates the protein level of Iqgap1 through ubiquitination and mediates the dynamics of focal complexes including the recruitment of paxillin and actinin. Loss of Hectd1 resulted in accelerated cell spreading but impaired directionality of migration and reduced β-catenin localization at adherens junctions, suggesting a molecular mechanism in which Hectd1 regulated the cell-cell contact and cell movements during neural tube development. In addition, we found that Hectd1 is a novel centrosome protein (Fig. 2) and it regulates centrosome duplication and disjunction. Hectd1 interacts with numbers of proteins in Y2H and MS assays, suggesting the eminant role of this gene in many basic cellular actions.

Within the frame of the Swiss Center of Applied Human Toxicology (SCAHT) we established a novel *in vitro* assay based on differentiating human embryonic stem cells (hESC) for testing early neurodevelopmental toxicity, focusing to stages corresponding to the formation of the neural tube and the generation and proliferation of the neural precursors. This model was setup as a proof of principle aiming at demonstrating that hESC can be used for developmental toxicity testing. We have confirmed the validity and reliability of our assay by analyzing the effect of three neuroteratogens: valproic acid (VPA), cyclopamine (CPA) and nicotine and two control compounds, the hepatotoxic but non-embryotoxic compound theophylline and the neutral compound saccharin, on four independent hESC lines. We have shown that our assay allows the specific identification of neurodevelopmental toxicants, can identify a developmentally toxic effect independently of effect on cell viability, allows an estimation of the toxic dose coherent with *in vivo* data, can distinguish effects of toxicants with different mode of action and different outputs *in vivo*, but does not allow to recognize, and therefore to predict, a particular *in vivo* defect. To determine the correct readout(s), we questioned the capacity of our system to recognize/predict a neural tube defect (NTD). We found that immunocytochemistry analysis cannot be used to reveal differential effects of VPA vs non NTD-inducing toxicants. However, we have selected a number of other markers, specifically and transiently expressed in rosette cells, whose expression can be quantified by RT-PCR. Dose/response experiments have been performed and analyses are ongoing to identify those with the optimal differential expression among the toxicants evaluated.

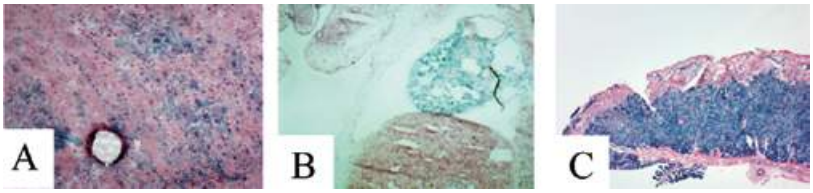

Fig. 1: Expression of Hectd1 in brain (A), embryonic heart at E13.5 (B) and placenta (C, E16.5).

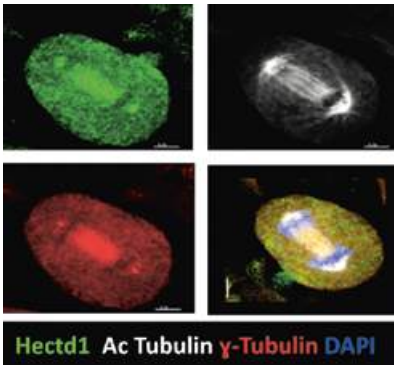

Fig. 2: Hectd1 is a novel centrosome protein

Selected Publications

Jia, Z., Gao, S., M'Rabet, N., De Geyter, C., Zhang, H. (2014) Sp1 is necessary for gene activation of Adamts17 by estrogen. Journal of Cellular Biochemistry, 115: 1829–1839.

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Cardiovascular Molecular Imaging

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Ultrasound molecular imaging in cardiovascular disease

Cardiovascular diseases are the leading cause of mortality in the western world. Most important are complications of atherosclerosis, but other disease entities such as myocarditis contribute to a considerable disease burden particularly in young individuals. Noninvasive imaging plays an increasing role in diagnosis, risk stratification and assessment of treatment responses. Advances in image technology over the last years have allowed for depiction of the heart and blood vessels with ever increasing detail. Novel imaging technologies termed molecular imaging use detection of site-targeted contrast agents to depict the molecular footprint of a disease-relevant phenotype at the cellular level. It is thought that such techniques will in the future contribute to earlier detection of disease, to better risk stratification and to better assessment of treatment responses. Molecular imaging with ultrasound contrast agents relies on the detection of microbubbles within diseased tissue. Microbubbles produce an acoustic signal owing to their resonant properties in an ultrasound field. Microbubble targeting is accomplished by either manipulating the microbubble shell for attachment of microbubbles to activated leukocytes, or by conjugation of specific ligands to the microbubble surface (Fig. 1).

Ultrasound molecular imaging of myocarditis

Dilated cardiomyopathy as a consequence of viral myocarditis is a frequent cause for heart failure in young adults. In young patients presenting to the emergency department with either chest pain or signs of heart failure, myocarditis is a differential diagnosis. However, the diagnosis of myocarditis is difficult, as clinical signs, the electrocardiogram and biomarkers (troponins) lack sensitivity/specificity. Thus, there is a need for a rapid, non-invasive imaging tool for the detection of inflammatory events occurring in myocarditis. Using microbubbles targeted to leukocytes, to CD4+ lymphocytes and to the endothelial cell adhesion molecule P-selectin we were able to diagnose myocardial inflammation in a murine model of myocarditis even in the absence of effects on myocardial function. The specific detection of the recruitment of CD4+ lymphocytes which are important in driving autoimmune processes that lead to cardiac damage in myocarditis was possible using non-invasive ultrasound molecular imaging. Also, the signals obtained from microbubbles targeted to CD4+ lymphocytes correlated to CD4+ lymphocytes present in tissue as assessed on immunhistology.

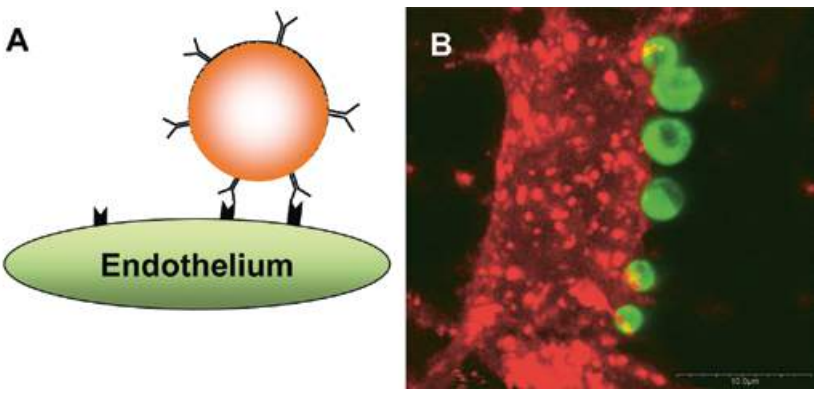

Fig. 1: Principle of site-targeting of microbubble contrast agents. **(A)** Antibodies or other ligands targeted to disease specific antigens are conjugated to the microbubble surface. **(B)** Attachment of microbubbles to VCAM-1 to an endothelial cell *in vitro*.

Ultrasound molecular imaging of atherosclerosis

Risk assessment for atherosclerosis relies on established clinical risk factors. This approach places a large proportion of individuals in an intermediate risk category. Therefore, tools to better assess the risk in these patients are needed. It is generally thought that noninvasive imaging of molecular events associated with atherosclerotic disease may serve this purpose. Previous studies have shown that contrast enhanced ultrasound (CEU) molecular imaging using microbubble contrast agents directed against vascular cell adhesion molecule 1 (VCAM-1), which is involved in inflammatory processes in atherosclerosis, is feasible in murine disease models. However, the ultrasound contrast agents used in these studies are not suitable for clinical translation, and there is a need for the development of microbubbles employing (a) clinically translatable strategies for conjugation of targeting moieties, and (b) targeting ligands that can readily be used in the clinical field. Nanobodies are small antibody fragments derived from heavy-chain-only antibodies. They are attractive for applications in molecular imaging, as they are highly specific, non-immunogenic and thus offer the potential for clinical translation. Likewise, Designed Ankyrin Repeat Proteins (DARPins) are potential candidates for clinical molecular imaging given their easy production and selection, high affinity and low immunogenicity. We are therefore currently developing and validating clinically translatable binders coupled to the microbubble surface using maleimide covalent bonding.

Selected Publications

Steinl M, Xu L, Khanicheh E, Ellertsdottir E, Ochoa-Espinosa A, Mitterhuber M, Glatz K, Kuster GM, Kaufmann BA. (2016) Non-invasive contrast enhanced ultrasound molecular imaging detects myocardial inflammatory response in autoimmune myocarditis. Circ Cardiovasc Imaging. Aug;9(8). pii: e004720

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Khanicheh E, Qi Y, Xie A, Mitterhuber M, Xu L, Mochizuki M, Daali Y, Jaquet V, Krause KH, Ruggeri ZM, Kuster GM, Lindner JR, Kaufmann BA. (2013) Molecular Imaging Reveals Rapid Reduction of Endothelial Activation in Early Atherosclerosis With Apocynin Independent of Antioxidative Properties.. Arterioscler Thromb Vasc Biol. Aug 1

Khanicheh E, Mitterhuber M, Xu L, Haeuselmann SP, Kuster GM, Kaufmann BA. (2013) Noninvasive Ultrasound Molecular Imaging of the Effect of Statins on Endothelial Inflammatory Phenotype in Early Atherosclerosis. PLoS ONE 8(3): e58761. doi:10.1371/journal.pone.0058761

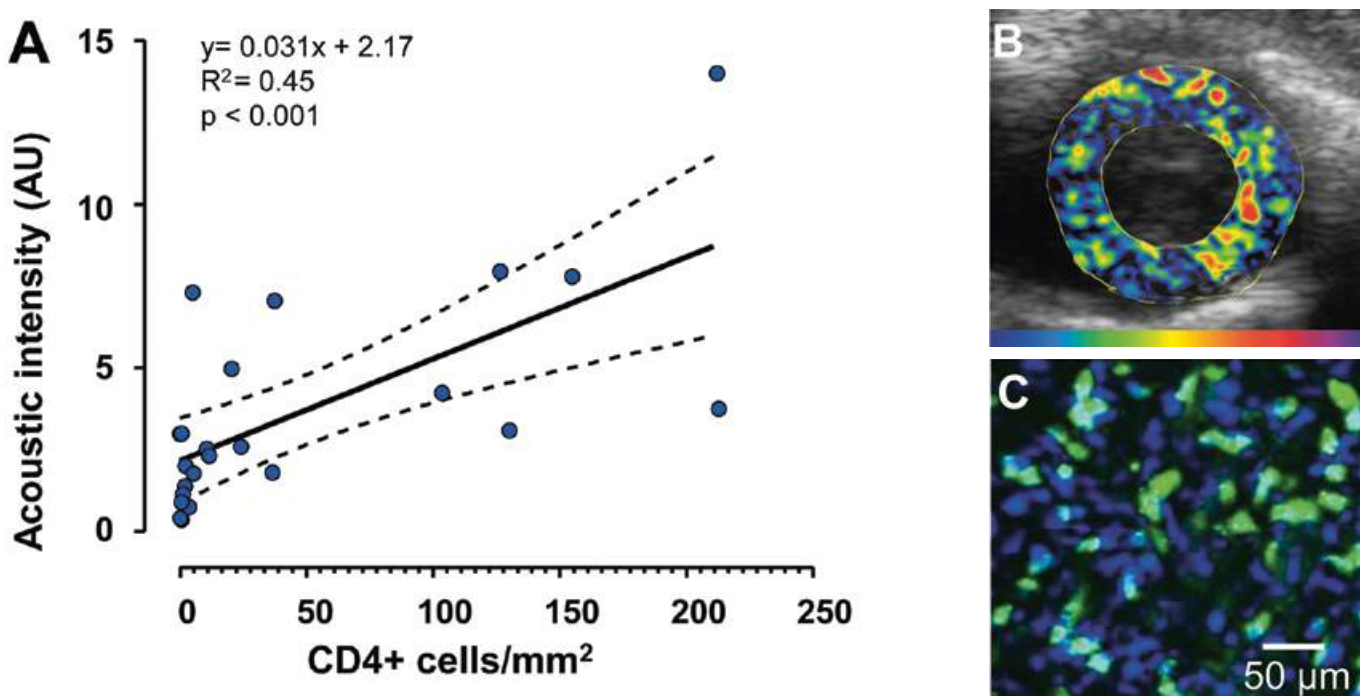

Fig. 2: Correlation of CEU molecular imaging data for CD4 targeted microbubbles with CD4+ T-lymphocyte counts in tissue **(A).** Example of background-subtracted signal for CD4 targeted microbubbles in an animal with myocarditis **(B).** Example of extensive myocardial CD4+ T-lymphocyte infiltration **(C).**

Clinical Pharmacology

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Idiosyncratic toxicity of drugs

Our lab is mainly engaged in the investigation of toxicological mechanisms of drugs and other chemical compounds on the liver, skeletal muscle and on the heart. During the last 3 years we published reports mostly in the fields of statin-associated myo- and cardiac toxicity and of hepatotoxicity associated with different drugs such as dronedarone, benzbromarone and flucloxacillin. All of these drugs are idiosyncratic toxicants; adverse effects are rare and appear at therapeutic doses, and only in patients with susceptibility factors. Principle aims of our studies are to understand the mechanism of toxicity and, based on the mechanism, to propose and investigate potential susceptibility factors.

Regarding statins and myotoxicity, we have shown that statins inhibit the AKT/mTOR pathway in C2C12 cells but also *in vivo* in mice. As a consequence, skeletal muscle protein synthesis is impaired, muscle breakdown is upregulated by induction of atrogin-1 and apoptosis of myocytes is increased. The reasons for the inhibition of AKT phosphorylation are inhibition of signaling across the IGF-1 receptor and inhibition of the activation of mTORC2. The IGF-1 receptor is N-glycosylated and N-glycosylation is impaired by statins. We have shown that in C2C12 cells, but have obtained similar findings in the heart and in skeletal muscle of mice treated with statins. In additional projects we could confirm that statins are mitochondrial toxicants and that they are associated with mitochondrial ROS production in cultured cells, mice and also in skeletal muscle of humans. Future studies will focus on answering the question why statins inhibit activation of mTORC2 and on the molecular mechanisms of insulin resistance associated with statins. Since insulin uses the same intracellular signaling pathway like IGF-1, it can be assumed that similar mechanisms are responsible for statin-associated insulin resistance. Furthermore, we are going to study the effect of impaired mitochondrial proliferation (using PGC1-α and PGC1-β knock-out mice) as a risk factor for statin-associated myopathy. Finally, we plan to investigate the mechanisms of statin-associated liver injury.

A second field of interest is the mechanism of hepatotoxicity associated with specific drugs. For that, we have studied the effects of benzbromarone and dronedarone on isolated liver mitochondria, on human liver cell lines and primary hepatocytes and in mice *in vivo*. Benzbromarone and dronedarone are mitochondrial toxicants which inhibit the function of the electron transport chain and of β-oxidation. *In vivo*, dronedarone was more toxic in mice with a defect in mitochondrial β-oxidation than in the corresponding wild type animals. This suggests that impaired mitochondrial β-oxidation may be a susceptibility factor for dronedarone-associated liver injury. Interestingly, dronedarone and benzbromarone break up the mitochondrial network, leading to fragmentation of the mitochondria (mitochondrial fission, see Fig. 1 and Fig. 2). They are also associated with increased mitochondrial production of ROS. Future studies will focus on the consequences of ROS production on the antioxidative defense system (Nrf2 activation and downstream reactions) and on the consequences of mitochondrial fission (increased mitophagy). Defects in activating the antioxidative defense system and/or in mitophagy could represent susceptibility factors for toxicity. This possibility will be tested in engineered cells and in mice. We will expand our research also on tyrosine kinase inhibitors, which are known mitochondrial toxicants.

Our studies show that, in contrast to what is written in many textbooks, it is possible to find mechanisms for idiosyncratic toxicity. This allows identifying susceptibility factors with the final aim to prevent this type of toxicity in patients.

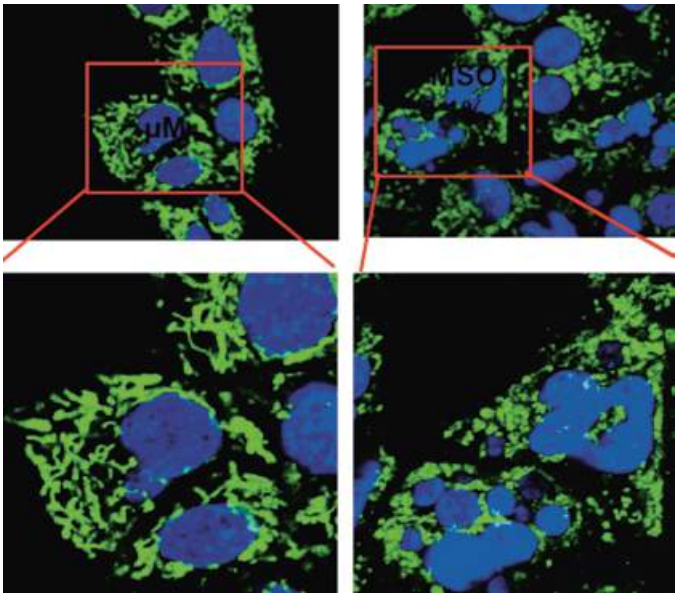

Fig. 1: Confocal microscopy of HepG2 cells treated for 24h with benzbromarone (BB) or with DMSO 0.1 % (control) and stained with an antibody against TOMM22. The mitochondrial network in cells treated with benzbromarone has a granular appearance, suggesting mitochondrial fission.

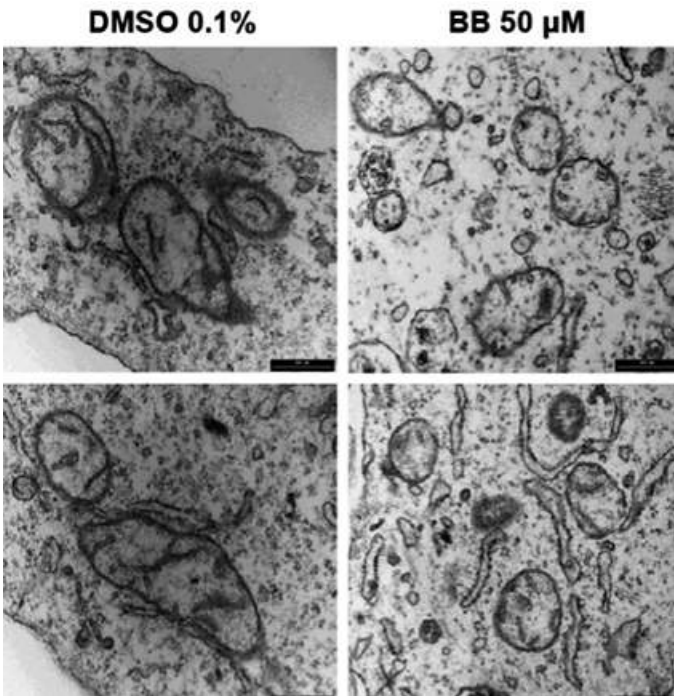

Fig. 2: Transmission electron microscopy of HepG2 cells treated for 24h with benzbromarone (BB) or with 0.1 % DMSO (control). Mitochondria in cells treated with benzbromarone appear smaller than in control cells, compatible with mitochondrial fission. The bar is 500 nm.

Connection to Clinical Practice

Modelling of organ toxicity of tyrosine kinase inhibitors

Tyrosine kinase inhibitors (TKIs) inhibit the phosporylation of proteins which are essential for cell proliferation. They have therefore become important for treating different types of cancer. During the development and clinical application of TKIs it was recognized that most of them carry the risk for organ toxicity, e.g. for liver, skeletal muscle and/or cardiac toxicity. Several recent reports have shown that the mechanism may be related to effects on mitochondrial function by these drugs. Since serum concentration and tissue distribution of these agents has been characterized, it would be possible to predict organ toxicity, if precise data about cellular toxicity were available. We plan to fill this gap and will therefore study organ toxicity of some of these drugs using established cell models and in mice *in vivo*. After having obtained these data, we will build a model which predicts organ concentrations and organ toxicity for individual TKIs in relation to dose and plasma concentrations in humans. Based on such data, we will be able to monitor patients treated with such drugs with the aim to individualize and optimize the dosing.

Selected Publications

Bonifacio A, Mullen PJ, Mityko IS, Navegantes LC, Bouitbir J, Krahenbuhl S. (2016) Simvastatin induces mitochondrial dysfunction and increased atro- gin-1 expression in H9c2 cardiomyocytes and mice *in vivo*. Arch Toxicol 90, 203–215

Bonifacio A, Sanvee GM, Bouitbir J, Krahenbuhl S. (2015) The AKT/mTOR signaling pathway plays a key role in statin-induced myotoxicity. Biochim Biophys Acta 1853, 1841–1849

Bouitbir J, Singh F, Charles AL, Schlagowski AI, Bonifacio A, Echaniz-Laguna A, Geny B, Krahenbuhl S, Zoll J. (2016) Statins Trigger Mitochondrial Reactive Oxygen Species-Induced Apoptosis in Glycolytic Skeletal Muscle. Antioxid Redox Signal 24, 84–98

Felser A, Blum K, Lindinger PW, Bouitbir J, Krahenbuhl S. (2013) Mechanisms of hepatocellular toxicity associated with dronedarone--a comparison to amiodarone. Toxicol Sci 131, 480–490

Felser A, Lindinger PW, Schnell D, Kratschmar DV, Odermatt A, Mies S, Jeno P, Krahenbuhl S. (2014) Hepatocellular toxicity of benzbromarone: effects on mitochondrial function and structure. Toxicology 324, 136–146

Myocardial Research

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Growth control and regeneration pathways in the heart

Heart failure ensues as the final common pathway of cardiac diseases, most frequently as a sequel of myocardial infarction. Despite improvements in patient outcome with state-of-the-art therapy, which includes neurohormonal modulation and devices, it still represents a leading cause of death and hospitalization. The recent recognition of intrinsic regenerative capacities in the adult heart that are thought to participate in organ homeostasis, but to be insufficient to compensate for cell loss after injury, provides now a basis for novel therapeutic strategies that aim at the strengthening of endogenous regeneration pathways and the use of cardiac progenitor cells (CPCs). A major interest of our laboratory is to decipher signaling and gene regulatory networks that are involved in tissue homeostasis and cell replacement in the heart, and to elucidate how they regulate the delicate balance between the proliferation of immature precursor and progenitor cells and their differentiation and maturation.

The microenvironment is an important regulator of cell fate and provides cues from the extracellular matrix (ECM) as well as soluble factors. We are particularly interested in how information from the ECM is transduced into a cell fate-regulatory response. We recently found that the differential regulation of YAP and of the cell cycle regulator Plk2 in response to ECM proteins either maintains proliferation of CPCs, thus contributing to their amplification, or directs them towards lineage commitment and differentiation, whereby Plk2 appears to act as an inverse link between cell cycle and fate decision. YAP and Plk2 are part of a developmentally regulated pathway, which is gradually shut down in the postnatal heart, thus allowing for terminal differentiation and maturation of cardiac cells. Such master regulators of cardiac cell fate could be therapeutically targeted to promote regeneration of the injured heart.

In a related line of studies with focus on the role of stem cell-regulatory factors in the heart, we recently uncovered an unexpected function of the hematopoietic cytokine Flt3 ligand and its receptor Flt3 as gatekeepers of CPC quiescence. We have previously shown that Flt3 is upregulated in the ischemically injured heart and that its pharmacological activation confers cytoprotection, hence improving remodeling and function after myocardial infarction. Flt3 is part of the cancer kinome and cardiomyopathies have been observed in patients under Flt3-targeting receptor tyrosine kinase inhibitor (TKI) therapy. Using functional analyses and relating them to whole transcriptome profiling, we are also studying how targeted cancer therapeutics, specifically TKIs, affect cardiac function. Results from these studies will not only provide a mechanistic rationale for cancer drug-related cardiotoxicity, but also help identify key effectors of cardiac growth and regeneration pathways.

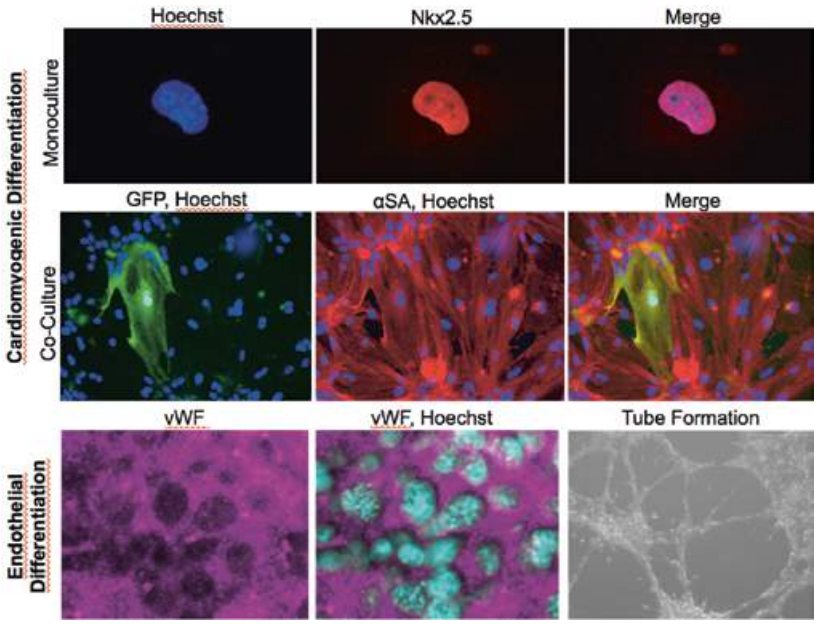

Fig. 1:
Cardiomyogenic and endothelial differentiation of cardiac progenitor cells (CPCs).
Top: Expression of cardiomyogenic transcription factors (shown Nkx2.5) after three weeks in monoculture. Expression of α-sarcomeric actinin (αSA) in a green-fluorescent protein (GFP)-expressing CPC-derived cell after three weeks in co-culture with cardiomyocytes. Bottom: Expression of von Willebrand Factor (vWF) protein after three weeks in endothelial differentiation medium. Such cells exhibit an endothelial cell phenotype with the capacity of tube formation in Matrigel.

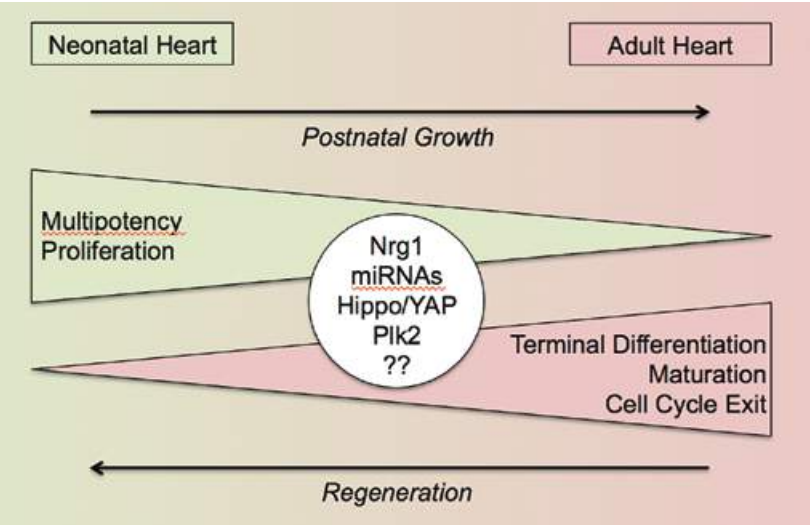

Fig. 2:
Relationship between regenerative capacities and organ/cell maturation in the heart.
The regenerative capacities of the mouse heart are lost within the first week after birth, when key pathways balancing cell cycle regulation and differentiation during development are shut down. Nrg1: neuregulin-1; miRNAs: micro RNAs; YAP: yes-associated protein; Plk2: polo-like kinase 2; ??: others and yet to be identified.

Selected Publications

Mochizuki M, Lorenz V, Ivanek R, Della Verde G, Gaudiello E, Marsano A, Pfister O, Kuster GM. (2016) Polo-like kinase 2 is dynamically regulated to coordinate proliferation and early lineage specification downstream of YAP in cardiac progenitor cells.

Kuster GM, Liao R. (2016) Fortune favors the prepared: Safety and efficacy of allogeneic hypoxia preconditioned mesenchymal stromal cells in primates. Circulation research 118, 908–910

Kuster GM, Della Verde G, Liao R, Pfister O. (2016) Cell therapy for cardiac regeneration. In Translating regenerative medicine to clinics, J. Laurence, M. Van Beusekom, P. Baptista, and A. Atala, eds. (United States of America: Academic Press, Elsevier Inc.), pp. 266–283

Pfister O, Della Verde G, Liao R, Kuster GM. (2014a) Regenerative therapy for cardiovascular disease. Translational Research 163, 307–320

Pfister O, Lorenz V, Oikonomopoulos A, Xu L, Hauselmann SP, Mbah C, Kaufmann BA, Liao R, Wodnar-Filipowicz A, Kuster GM. (2014b) FLT3 acti- vation improves post-myocardial infarction remodeling involving a cytoprotective effect on cardiomyocytes. Journal of the American College of Cardiology 63, 1011– 1019

Stem Cells and Hematopoiesis

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Stem cell pathways in development and oncogenesis

Various tumors have been shown to contain subpopulations of so-called cancer stem cells (CSCs), which are thought to be responsible for disease initiation, maintenance, metastasis and relapse after conventional anti-tumor therapies. We hypothesize that pathways that regulate stem cells during development can reactivate expression during oncogenesis and specifically in CSCs. For example, we show that enhanced expression of the pluripotency-related embryonic protein SOX2 associates with stemness, disease aggressiveness and therapy resistance in putative ovarian and breast CSCs. In breast carcinoma, SOX2 protein expression strongly relies on pAKT activity, suggesting AKT-inhibitors as promising drugs for targeting SOX2-expressing CSCs.
In our developmental studies, we previously identified the BMP-WNT-CDX-HOX signaling pathway as an essential regulator of embryonic hematopoiesis. Later on, involvement of CDX genes was demonstrated in human leukemia. Gene expression arrays performed on CDX2-modified leukemic cells confirmed HOX genes as targets but also revealed the transcription factor EVI1 (Ecotropic viral integration site 1) as a putative downstream molecule. EVI1 has been mostly studied in acute myeloid leukemia (AML), where high expression predicts adverse clinical outcome. We showed that EVI1 also expresses in lymphoblastic leukemia cells where it regulates apoptosis sensitivity, and furthermore plays important roles as an oncogene in breast as well as in prostate carcinoma, where it regulates cell growth and migration independently of estrogen and HER2 signaling, and respectively appears to control disease progression and therapy resistance at the stem cell level.

Using zebrafish to study hematopoiesis and tumor biology

EVI1 also plays important roles during development, amongst other regulating nascent hematopoietic stem cells (HSCs). Using *in vivo* live imaging studies on transgenic zebrafish embryos, we could show that evi1 suppression impairs HSC emergence by altering Notch levels in endothelial cells of the ventral dorsal aorta (VDA; the fish equivalent of the mammalian aorta-gonado-mesonephros region) and thereby impairing their transition to hematopoietic fate (Konantz *et al.,* 2016). Zebrafish are also used to xenograft human tumor cells and monitor tumor-induced angiogenesis, invasiveness, and response to a range of treatments *in vivo* and in real time. Moreover, our laboratory aims to generate transgenic zebrafish leukemia models by influencing EVI1, RAS and p53 expression in specific cell types of the hematopoietic compartment; these models are currently being characterized in detail on the functional and molecular levels and in the near future shall be used for drug screens. Finally, in a project sponsored by the Roche Postdoctoral Program (RPF), we recently showed that double transgenic gata1/globin zebrafish can be used to model erythroid lineage toxicity and regeneration; a small molecule screen is planned to further validate the model for a screening setting and to potentially identify new compounds that interfere with erythroid regeneration and maturation.

Characterization of murine AML xenotransplantation models

Repopulation of immunodeficient mice remains the primary method to functionally assess human AML. We recently developed in our laboratory an experimental approach that enables engraftment of ~95% of AML (instead of 40–60% as reported by previous studies), especially also successful with disease subtypes so far considered non-engraftable. We show that this model faithfully mimics human disease, since xenogeneic human AML cells derived from engrafted mice retain immune phenotypic and genetic characteristics of corresponding pre-transplant patient samples. Importantly, molecular risk subgroups established in patients were shown to predict time to engraftment/leukemia also in mice (Fig. C–E). We currently use this model to understand processes governing leukemia initiation.

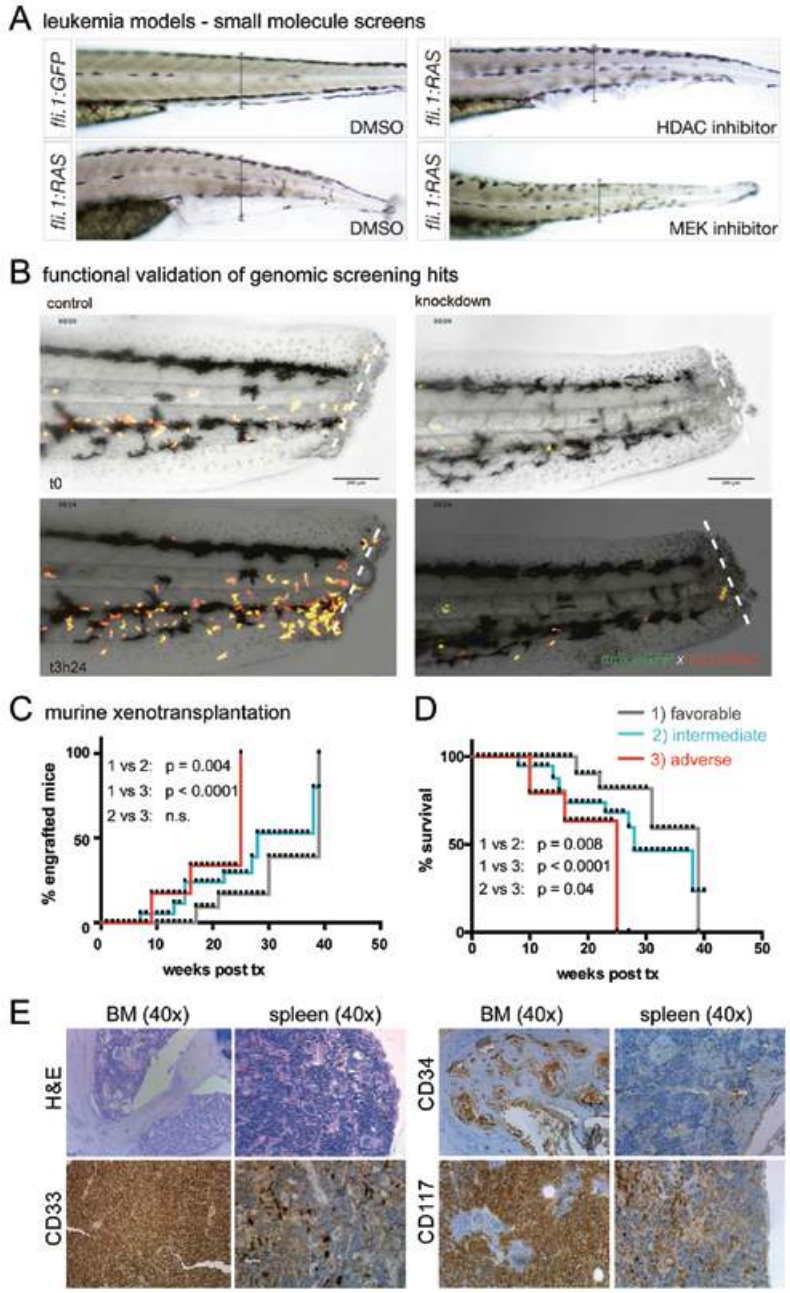

Figure: Using xenograft and zebrafish models to model human disease and identify new treatments.

A. Small molecule screens in zebrafish leukemia models. In transgenic *fli.1:RAS* fish (Alghisi *et al.* 2013), the caudal hematopoietic tissue – the equivalent to the mammalian fetal liver – is expanded due to malignant transformation. Phenotypic analysis after treatment with small molecules allows identifying novel compounds that can be used to target RAS induced leukemia.

B. Functional validation of genomic screening hits. Gene X was identified by whole exome screening as a novel mutation in neutropenia. Shown are two representative time points for control (left) and knockdown (right) transgenic Tg*(mpx:eGFP;lyz:DsRed)* embryos in which the migration of double positive neutrophils to the wound area is visible over time. For each time point, merged images are shown. Dotted lines indicate the localization of the tail fin amputation.

C–D. Human AML engraft NSG mice with latency depending on the molecular risk group of the xenotransplanted AML. Favorable risk AML, which in patients associates with improved survival rate, requires longer latency to engraft and induce leukemia in NSG mice, when compared to intermediate or adverse risk AML.

E. Leukemia induction in mice faithfully mimics human disease showing bone marrow (BM) and organ infiltration with leukemic cells with conserved expression of leukemic antigens. (From Paczulla *et al.*, 2016).

Connection to Clinical Practice

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- Investigating the relevance of selected mutations and oncogenic pathways using patient samples.
- Characterizing molecular mechanisms that mediate therapy resistance in cancer patients and identifying strategies to overcome them and could be tested in future clinical trials.
- Functional investigation of the relevance of mutations identified by genomic screens using the zebrafish model.

Selected Publications

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Paczulla AM, Dirnhofer S, Konantz M, Medinger M, Salih HR, Rothfeder K, Tsakiris D, Passweg J, Lundberg P and Lengerke C. (2016) Long-term observation reveals high frequency engraftment of human myeloid leukemia in immunodeficient mice. Haematologica

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Development and Evolution

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Functional Genomics of Limb Development: Mechanisms of Morphological Evolution and the Link to Human Congenital Malformations

A fascinating challenge in biology is to unravel the mechanisms underlying the evolutionary diversification of animal form. The vertebrate limb skeleton is a particularly useful model system to do so, as its anatomy has been drastically altered in evolution through adaptive changes to different modes of life and locomotion. The diversification of anatomies seen in tetrapods derives from the modification of a common ancestral limb skeletal *bauplan* that had five digits. So far, expression studies suggest that the same set of developmental networks is involved in the morphogenesis of the limb in all tetrapods. So… how did all these morphologies arise in evolution? The current view in evolutionary developmental biology (evo-devo) is that form evolves through changes in the level or spatial-temporal pattern of expression of pleiotropic developmental genes. In a nutshell, this means that the mutations that drive the divergence of morphologies mostly affect the regulatory regions in the genome that control the transcription of highly conserved developmental genes, rather than the coding sequences themselves.

Artiodactyls constitute a paradigmatic case of limb specialization, as they display skeletal adaptations for running on uneven terrain, such as elongated limbs and digit reductions/loss. In particular, bovine limbs have only two toes of equal length, allowing them to walk on the distal-most phalanges. We have established that AP polarity is progressively lost in the handplate mesenchyme during bovine limb bud development. This leads to distal restriction of the digit-forming domain and the preferential elongation of the two symmetric digits that will form the hoofed toes. We have identified that failure to sense graded SHH signaling underlies the loss of handplate asymmetry in bovine embryos. In particular, the expression of the SHH receptor PTCH1 is not upregulated in the bovine limb bud mesenchyme. Using functional genomic approaches, we identified a SHH-responsive limb enhancer of *Ptch1* that we termed LRM and that has functionally degenerated in the bovine lineage. This study provides a molecular and mechanistic explanation for evolutionary digit loss in artiodactyls and illustrates how changes in developmental gene regulatory networks contribute to the appearance of anatomical novelties in evolution (Fig. 1)

As a continuation of these studies, we are currently using the CRISPR/Cas9 technology to modify the mouse genome and sequentially delete the different conserved regions within the *Ptch1* LRM enhancer. These studies, in parallel with a systematic analysis of the entire *Ptch1* cis-regulatory landscape using transgenic reporter embryos will allow us to understand how SHH signaling is integrated at the transcriptional level in a tissue-specific manner.

In addition, we are also studying limb development in another artiodactyl, the pig. In these studies, we are using ATAC-seq and RNA-seq in pig and mouse limb buds to identify diverging regulatory strategies affecting key developmental genes. This approach is allowing us to gain mechanistic knowledge into the gene regulatory networks operating in limb development and uncovering how they may have been rewired during the evolution of vertebrate limb morphology. Most importantly, by studying how form is genetically encoded, our goal is to gain fundamental insight into the human mutations leading to congenital malformations of the limb. As it is estimated that a vast proportion of disease-causing mutations map to the regulatory genome, we are sequencing, in collaboration with clinical geneticists, some of these candidate cis-regulatory regions in patients with congenital limb defects.

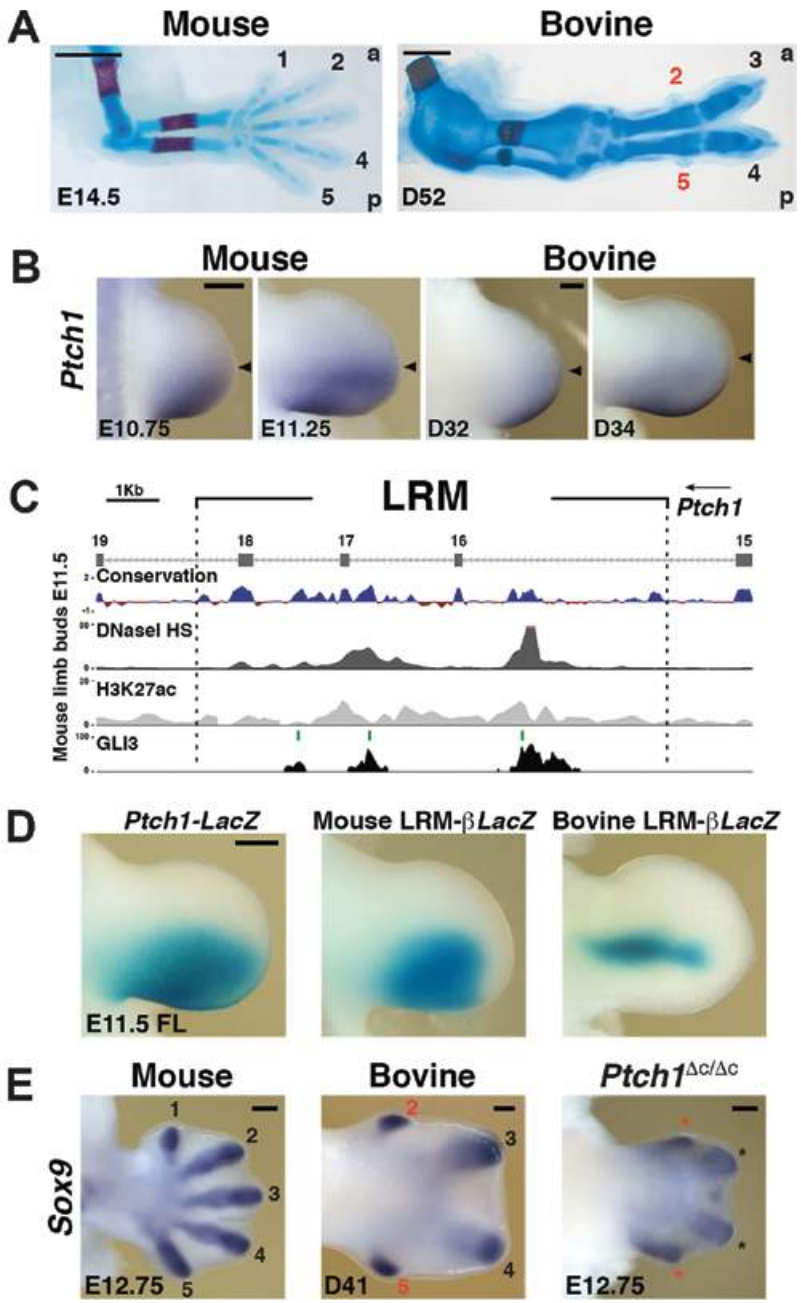

Fig. 1: Mechanisms of evolutionary diversification of the bovine limb skeleton. A) The bovine handplate is symmetric and has only two digits, while the mouse limb is pentadactylous and displays clear anterior-posterior polarity. a: anterior; b: posterior. B) *Ptch1* is not upregulated in the distal mesenchyme in bovine limb buds. C) The *Ptch1* cis-regulatory module (LRM) is located within the exonic structure of the *Ptch1* gene and displays characteristics of a *bona fide* enhancer that responds to SHH, such as open chromatin, H3K27ac marks and occupancy by GLI transcription factors. D) While the mouse LRM (middle panel) drives *lacZ* reporter gene expression in a pattern similar to that of the endogenous gene (left panel), the bovine LRM has functionally degenerated (right panel). E) The genetic inactivation of *Ptch1* in the mouse limb bud mesenchyme (right panel) reproduces a digit pattern that is reminiscent of the one observed in cattle (middle panel). See Lopez-Rios *et al.*, 2014 for details.

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Cardiac Surgery and Engineering

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*left during report period

3D Engineered tissues as angiogenic therapeutic approach and as functional cardiac models

Research Summary: The ultimate goal of the research group is to investigate angiogenic therapies based on engineered tissues (patches) to induce safe and efficacious angiogenesis in a chronic ischemic myocardium. For this purpose two different strategies are currently investigated relying on the use of either (1) VEGF-expressing cells or (2) heterogeneous cell population with a high angiogenic potential. The group also aims to (3) develop *in vitro* functional cardiac models to investigate processes of myocardial repair/regeneration. Research is funded by the Swiss National Science Foundation and Swiss Heart Foundation.

Patches engineered by VEGF-expressing cells. Therapeutic angiogenesis induced by exogenous VEGF delivery is a promising strategy. However, VEGF release at the microenvironmental level needs to be controlled to induce only normal capillary network, avoiding growth of aberrancies. A FACS-based technique was used to purify transduced human adipose tissue-derived mesenchymal stromal cells (ASC) that homogeneously express a safe VEGF dose from a heterogeneous primary population (Helmrich U, 2011) (collaboration with A. Banfi). Direct intramyocardial injections of ASC expressing safe VEGF-levels induced controlled angiogenesis in the heart, beside the poor cell survival observed (Melly and Marsano, 2012). Thereafter, skeletal myoblasts expressing safe VEGF levels co-cultured with cardiomyocytes in 3D scaffolds were shown to induce an efficacious angiogenesis in engineered cardiac tissues and superior cell survival upon implantation into an ischemic myocardium (Marsano, 2013). We then hypothesized that patches generated by controlled VEGF-expressing ASC could induce normal and efficient angiogenesis not only in the patch itself but also in the surrounding area, working as a controlled delivery system. We found that VEGF release induced normal angiogenesis in the patch already after 7 days, and in the surrounding avascular area (simulated by an empty 7mm-thick cell-free collagen sponge) after 28 days upon implantation in a subcutaneous rat model (Fig. 1). Patch prompt vascularization resulted in an increased survival of implanted cells up to 28 days (Boccardo and Gaudiello, 2016).

Patches engineered by cells with a high angiogenic potential. In this approach, we used human adipose tissue-derived stromal vascular fraction (SVF) cells as a heterogeneous cell population with a high angiogenic potential thanks to the presence of numerous endothelial/mural progenitors (collaboration with A. Scherberich). We hypothesized that perfusion-based bioreactor culture supported the maintenance of endothelial/mural cells as compared to static culture, thereby accelerating the whole construct vascularization and supporting the cell survival upon implantation in a subcutaneous rat model. Our findings showed that perfusion-based culture significantly modulated the initial SVF cell population composition, leading to a significant enrichment of the pericytes compared to static condition. The enriched perfusion-based engineered constructs showed an accelerated *in vivo* vessel ingrowth at 3 days and promoted the formation of blood vessels by cells of human origin.

3D functional cardiac models. Our angiogenic engineered tissues might also affect cardiac repair/regeneration by influencing cardiomyocyte maturation and functionality and progenitor cell recruitment. Therefore, we aimed here to generate 3D functional cardiac models as tool to investigate interactions of VEGF-expressing ASC/SVF cells and cardiomyocytes. We hypothesized that the recapitulation of the proper physiological conditions, mimicking the native tissue environment, enhanced the cardiomyocyte maturation, 3D organization and functionality. Culture medium perfusion systems were employed to mimic the highly dense capil-

lary network present in the myocardium to ensure the cardiomyocyte survival *in vitro* (Marsano, 2010; Maidhof, 2010; Cerino, 2016). Mechanical stimulation (collaboration with the Politecnico of Milano, Italy) was employed to greatly promote human induced pluripotent stem cell-derived cardiomyocyte maturation and contractility (Marsano, 2016).

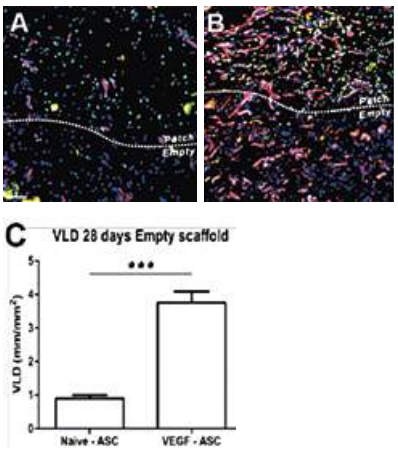

Fig. 1: Representative immunofluorescence images of border between the empty collagen scaffolds and the patches generated by naïve (A) or VEGF-expressing ASC (B) after 29 days *in vivo* (Ve-Cadherin in red; human specific nuclei in green; "staining with" DAPI for cell nuclei in blue). Size bar = 100µm. Graph represents the vessel length density (VLD) assessed in the empty scaffolds after 28 days *in vivo.* Data are represented as mean ± SEM. (n = 3) (B).

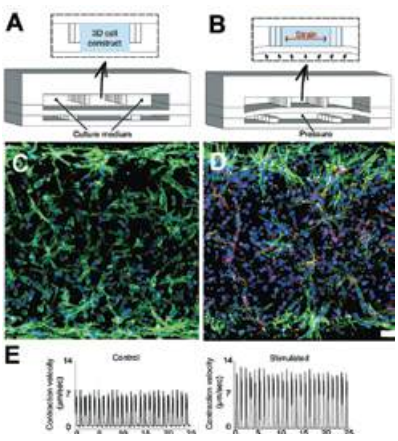

Fig. 2: Design of the 3D heart-on-a-chip microdevice (A-B). Constructs (A) consisted of cardiomyocytes embedded in fibrin gel. By pressurizing the bottom compartment (pressure, B) the PDMS membrane deforms, compressing the 3D cell construct (strain, B). Effects of cyclic strain was evaluated after 5 days in culture by the expression of specific cardiac markers (cardiac Troponin I in green; connexin-43 in red; DAPI used for cell nuclei in blue, C-D) and by the contraction rate during spontaneous beating (control and stimulated micro-tissues E).

Selected Publications

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Marsano A, Medeiros da Cunha CM, Ghanaati S, Gueven S, Centola M, Tsaryk R, Barbeck M, Barbero A, Helmrich U, Schaeren S, *et al.* (2016) Spontaneous *in vivo* chondrogenesis of bone marrow-derived mesenchymal progenitor cells by blocking VEGF signaling. Stem Cells Trans Med in Press

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*Corresponding Authors

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Connection to Clinical Practice

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Engineered tissues with high angiogenic potential to treat chronic cardiac ischemia

Chronic myocardial ischemia causes progressive deterioration of cardiac function and may lead to end-stage heart failure. However, if blood flow is restored, the tissue at the border zone is capable of resuming full function. Surgical revascularization strategies are currently used to re-establish the macro-circulation. However, some patients could also benefit from an adjuvant pro-angiogenic/repair therapy, which aims at promoting the growth of microcirculation and at rescuing the damaged cardiomyocytes. The ultimate goal of the collaboration with the Cardiac Surgery is to investigate a cell-based therapy capable to induce safe, efficacious angiogenesis in a chronic ischemic myocardium. The strategy here pursued is based on the tissue engineering paradigm which provides control over the targeted area -reducing not desired systemic effects- and superior implanted cell survival compared to cell intramyocardial injection delivery. The current research program of the group includes the engineering of 3D patches with high angiogenic potential made by human adipose tissue-derived stromal vascular fraction cells, known to (1) contain subpopulations of both mesenchymal and endothelial progenitor cells and (2) release a broad range of pro-cell-survival factors. Specific induction of microvascular networks and release of cardioprotective factors in the hypo-perfused myocardial areas might be crucial to preserve cardiomyocyte survival and rescue their contraction capability in order to improve the overall cardiac function.

Tissue Engineering

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From 3D culture models to regenerative surgery

The common denominator of the research projects in the group is related to the establishment of 3D cell culture systems, combining interdisciplinary efforts in cell biology, engineering technologies and materials science. These systems are used as models to investigate fundamental aspects of tissue development, and as grafts to induce tissue regeneration. Several collaborations have been established within the DBM to employ the developed tools for 3D culture of tumor cells (Prof. G. Spagnoli, Prof. G. Iezzi), endothelial cells (PD Dr. A. Banfi), thymic epithelial cells (Prof. G. Holländer), glial cells (Prof. R. Guzman), pluripotent stem cells (Prof. C. De Geyter) and cardiac cells (PD Dr. A. Marsano). However, the main focus has been maintained around the development of cartilage and bone/bone marrow tissues. Following is a short summary of recent achievements in these research areas.

Nasal chondrocytes and environmental plasticity (PD Dr. A. Barbero)
We found that chondrocytes from the nasal septum, as compared to articular chondrocytes typically used for cell-based cartilage repair, have superior and more reproducible chondrogenic capacity. This led to the first clinical trial demonstrating the suitability and safety of engineered nasal cartilage grafts for reconstructive purposes in the nose. We also found that nasal chondrocytes – of neural crest origin – display a distinct profile of HOX gene expression from articular chondrocytes – of mesoderm origin. However, upon implantation in a joint, they can be reprogrammed by the recipient site and acquire the HOX “signature” typical of articular chondrocytes. These results have led to the treatment of 17 patients with traumatic cartilage injuries in the knee (Fig. 1). Positive clinical findings have been instrumental to receive EU funding for a multicenter phase II study.

Bone, Bone marrow and Vascularized bone (PD Dr. A. Scherberich)
The use of mesenchymal stromal/stem cells (MSC) from bone marrow has been proposed to generate osteogenic grafts, but with limited efficiency and reproducibility. We hypothesized that the robustness of the process could be increased by mimicking events of bone embryonic development. Intermediate templates of hypertrophic cartilage generated by MSC could robustly and autonomously remodel into bone tissue. A similar outcome could also be achieved with engineered and decellularized tissues, where the cocktail of factors necessary to initiate the process is embedded in the deposited extracellular matrix. The bone organ developed by this approach is capable to host fully functional hematopoietic stem cells and is thus being investigated in collaboration with Prof. R. Skoda and Prof. C. Lengerke as a humanized environment to study interaction of leukemic cells with a 3D stromal niche. MSC are also found within the Stromal Vascular Fraction (SVF) of adipose tissue, mixed with endothelial lineage cells. After demonstrating that SVF cells can self-assemble into osteogenic and vasculogenic structures, we used them in an intraoperative clinical setting to enhance humerus fracture healing in elderly patients (Fig. 2). Studies are ongoing to combine the strategy of engineered and decellularized matrices (see above) with intraoperative “re-activation” by SVF cells to enhance bone and vascular repair.

Engineering platforms (Dr. D. Wendt)
One main challenge in engineering 3D culture models or tissue grafts is to ensure efficient nutrition and oxygen supply through thick constructs. This has been addressed by developing bioreactor systems to perfuse cell suspensions/culture medium directly through the developing structures. The devices are used in a variety of settings for lab discovery, and are being further developed for the streamlined GMP manufacturing of cellular grafts for clinical use. More recently, we have also validated a microfluidic system allowing the formation of 3D cellular struc-

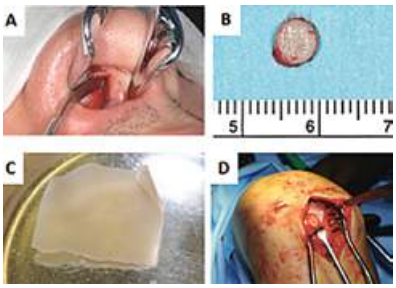

Fig. 1: Engineering of autologous nasal cartilage grafts. (A) Collection of a nasal cartilage biopsy from a patient, this procedure is performed under local anaesthesia and results in minimal donor site morbidity. (B) Biopsy of nasal cartilage septum. (C-D) Tissue engineered cartilage graft: macroscopic (C) and histological appearance (Safranin-O staining specific for sulfated glycosaminoglycans (D).

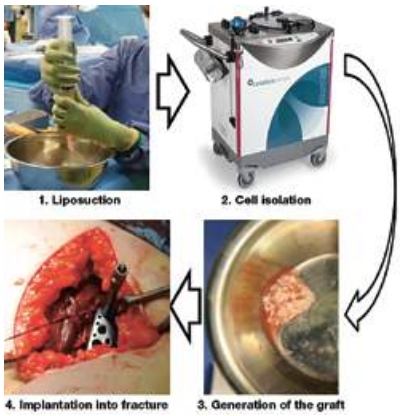

Fig. 2: Intraoperative engineering of autologous Stromal Vascular Fraction-based grafts for the clinical treatment of humerus fractures in osteoporotic individuals.

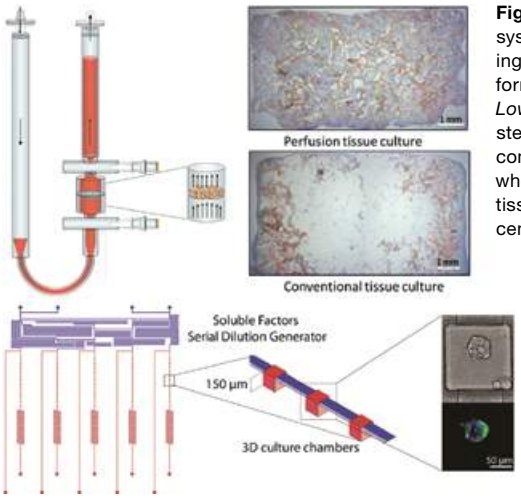

Fig. 3: *Upper panel:* Direct perfusion system to support efficient cell seeding into 3D porous scaffolds and uniform *in vitro* tissue development. *Lower panel:* Microfluidic-based system to allow high-throughput test of compounds within culture chambers, where cells can organize into microtissues and monitored by fluorescence microscopy online.

tures and their exposure to different combinations and concentrations of regulatory molecules. The system is in use to investigate in a relatively higher throughput the signals supporting the recapitulation of developmental programs by mesenchymal progenitors (Fig. 3).

Selected Publications

Mumme M, Barbero A, Miot S, Wixmerten A, Feliciano S, Wolf F, Asnaghi MA, Baumhoer D, Bieri O, Kretzschmar M, *et al.* (2016) Nasal chondrocyte-based engineered autologous cartilage tissue for the repair of articular cartilage defects: an observational first-in-human trial. Lancet 388, 1985–1994.

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Connection to Clinical Practice

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Orthopaedics and traumatology,
Plastic, reconstructive, aesthetic and hand surgery,
Spine surgery

Engineered grafts in trauma, orthopedic, spinal, plastic, reconstructive and maxillo-facial surgery

The main goal is the translation of engineered cellular implants into specific surgical procedures and reconstructive indications. These include the following directions.

Facial cartilage reconstruction. Engineered nasal cartilage grafts, which have been previously suvccessfully used for reconstruction of the alar lobule of the nose, are currently being investigated for the reconstruction of the nasal cartilage septum after perforation (e.g., due to leishmaniosis) (PD Dr. M. Haug, Dr. I. Fulco).

Articular cartilage and intervertebral disc repair. Following the demonstration of feasibility and safety of nasal chondrocyte-based engineered cartilage for the treatment of knee cartilage injuries, a phase II study enrolling 108 patients in 4 international centers has been financed by the Horizon 2020 EU Program to investigate the role of tissue maturation on the clinical outcome (Dr. M. Mumme, PD Dr. Pagenstert). Pre-clinical studies are also planned to explore the use of nasal chondrocytes to block degeneration of intervertebral discs in spinal surgery (Dr. A. Mehrkens)

Bone augmentation. Treatment of humerus fractures in elderly individuals indicated the safety and biological functionality of stromal vascular fraction (SVF) cells intraoperatively derived from autologous adipose tissue (Dr. F. Saxer, Dr. P. Studer). The combination of SVF cells with synthetic or engineered matrices is being investigated for vascularized bone graft prefabrication in the reconstruction of the upper jaw , for the treatment of avascular necrosis and for maxillary sinus augmentation (Dr. T. Ismail, Prof. C. Jaquiery, PD Dr. Alexandre Kämpfen, Prof. C. Kunz, Dr. R. Osinga, Dr. F. Thieringer)

Myeloid Malignancies

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Mechanisms and targeting of oncogenic signaling in myeloproliferative neoplasms

Myeloproliferative neoplasms (MPN) are chronic leukemias with excessive proliferation of mature myeloid cells. They present as essential thrombocythemia (ET) with thrombocytosis, polycythemia vera (PV) with erythrocytosis, or myelofibrosis (MF) with expansion of megakaryocytes and bone marrow fibrosis. They lead to bone marrow failure or may transform to acute myeloid leukemia (AML). Hematopoietic stem cell transplantation is the sole curative therapy, but is limited to a subset of patients. It is the goal of our studies to contribute to novel therapeutic approaches for MPN patients by targeting the molecular signaling driving these diseases. MPN are characterized by hyperactive signaling of the JAK2 kinase due to acquired mutations in the JAK2 signaling pathway. JAK2 is an intracellular non-receptor tyrosine kinase essential for hematopoiesis representing the exclusive mediator of signaling from the thrombopoietin receptor MPL, the erythropoietin and GM-CSF receptors. JAK2 activates several signaling pathways including STAT transcription factors, the phosphoinositide-3 kinase (PI3K) pathway and the mitogen activated protein kinase (MAPK) pathway, which promote cell proliferation, differentiation and survival. In MPN, JAK2 signaling is constitutively activated by mutations in JAK2, MPL or the chaperone protein Calreticulin. The central role of JAK2 signaling in MPN has led to the development of JAK2 inhibitors which act as ATP mimetics and stabilize JAK2 in the active conformation (type I inhibition, e.g. ruxolitinib). However, type I JAK2 inhibitors have not met the expectations. They fail to reduce the mutant clone suggesting limited curative potential, and induce resistance. To improve therapeutic targeting of JAK2 signaling in MPN, we are pursuing several approaches:

More effective targeting of JAK2
A new mode of JAK2 inhibition has recently been reported which stabilizes the inactive form of JAK2 (type II inhibition). We found high potency of type II JAK inhibition in preclinical MPN models. We observed decreased mutant allele burden due to preferential inhibition of mutant JAK2 suggesting type II inhibition could lead to a class of agents with curative potential. Resistance to type I JAK inhibitors is also abrogated. We are continuing our studies on type II JAK2 inhibition, which appears to herald the development of mutant-selective inhibitors, as a basis for improved therapeutic options.

Resistance mechanisms to JAK2 inhibitors
Response to type I JAK inhibitors is often lost upon prolonged exposure. JAK2 resistance mutations occur *in vitro*, but have not been observed in patients. It has been shown that MPN cells functionally adapt and reactivate JAK2 signaling through formation of JAK2 heterodimers with other JAK family members such as JAK1 and TYK2. We found that this escape mechanism extends to type I JAK2 inhibitors in clinical development, and observed cross-resistance. These molecular studies of resistance mechanisms to JAK inhibitors may reveal new therapeutic targets, while studies on patient samples will provide insight into clinical JAK2 inhibitor resistance.

JAK2 signaling network
JAK2 induces activation of STAT-, PI3K- and MAPK signaling. Therapeutic targeting of these pathways in other malignancies was impeded by feedback or cross-talk signaling revealing intricate signaling networks. We could show that combined inhibition of several targets such as JAK2 and Bcl-2/Bcl-xL can provide superior benefit in JAK2-driven leukemias. We are investigating the signaling network

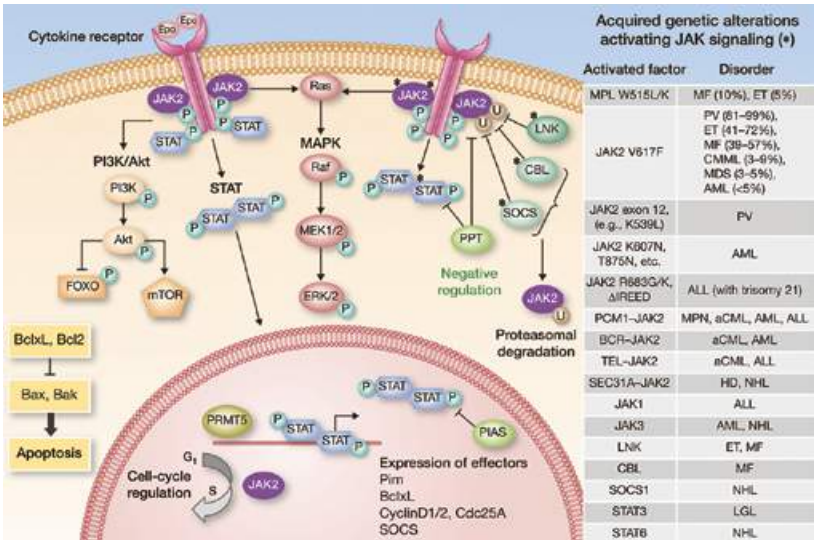

Fig. 1: Overview of JAK2 signaling. The JAK2 tyrosine kinase associates with hematopoietic cytokine receptors for EPO, TPO and GM-CSF. Upon ligand binding, JAK2 activates several signaling pathways including the STAT3 and STAT5 transcription factors, the PI3K/Akt pathway and the MAPK signaling pathway which includes RAS and the kinases RAF, MEK1/2 and ERK1/2. In MPN, JAK2 is constitutively activated by somatic mutations leading to excessive myeloid proliferation. The molecular interconnections between JAK2 and the downstream signaling pathways is not fully clarified. (Adapted from Meyer S.C. & Levine R.L., Clin. Cancer Res. 2014)

downstream of JAK2 to delineate the mechanisms limiting efficacy of JAK inhibitors and to inform novel therapeutic strategies. We aim to extend these studies to other myeloid malignancies with suboptimal clinical benefit of tyrosine kinase inhibitors.

JAK2 signaling in thrombopoiesis

Efficacy of therapeutic targeting with JAK2 inhibitors can be limited by on- and off-target toxicities. Thrombocytopenia is a significant side effect of JAK2 inhibition. We have shown that JAK2 regulates megakaryopoiesis including formation of megakaryocyte-biased stem cells, and are interested in differential effects of JAK2 inhibitors on thrombopoiesis.

Selected Publications

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Connection to Clinical Practice

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Characteristics of oncogenic signaling, therapeutic response and resistance in MPN patients

Our studies on oncogenic signaling and targeted therapeutic approaches in MPN are in close collaboration with Prof. R. Skoda who has established a long-term MPN patient cohort at University Hospital Basel, and Prof. J. Passweg, Head of the Division of Hematology at University Hospital Basel. We are studying clinical isolates of MPN patients with different mutational setup or at different stages of the disease for characteristics of oncogenic signaling and functional capacity of hematopoietic stem/progenitor cells. We are interested in the signaling dynamics in response to different therapies and upon development of resistance to conventional JAK2 inhibitors such as ruxolitinib. Collaborative studies with Prof. R. Levine based on the MPN cohort at Memorial Sloan Kettering Cancer Center New York, support these efforts. We aim to correlate the molecular findings with clinical characteristics of response or resistance to therapy in these MPN patients. These translational studies will facilitate potential clinical studies on improved targeting of JAK2 signaling in MPN in the longer term.

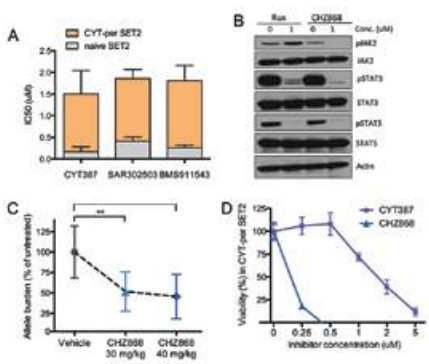

Fig. 2: Type II JAK2 inhibition provides a novel mode for improved targeting of JAK2 signaling in MPN. A. Conventional (type I) JAK2 inhibitors like CYT387 (CYT) induce cross-resistance in SET2 MPN cells. **B.** Type I JAK inhibitors like ruxolitinib (Rux) stabilize JAK2 in the phosphorylated form. The new type II JAK inhibitor CHZ868 stabilizes inactive, unphosphorylated JAK2. **C.** Type II JAK2 inhibition reduces mutant allele burden in MPN *in vivo* models. **D.** Type II JAK inhibition abrogates type I inhibitor resistance, shown for CYT387 resistant SET2 cells (Adapted from Meyer S.C. et al, Cancer Cell, 2015).

Musculoskeletal Research

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Form-Function relationship in the musculoskeletal system

The glenohumeral joint – a mismatching system? A morphological analysis of the cartilaginous and osseous curvature of the humeral head and the glenoid cavity

Radial mismatch, glenohumeral conformity ratios and differences between cartilaginous and osseous radii highly depend on the measured plane. The comparison of cartilaginous radii between humeral head and glenoid in different planes provides new information to understand the degree of conformity during abduction of the upper limb. To investigate the radii, CT-images of shoulder specimen were analysed using an image visualization software and statiscically analysed. Measurements of the radii in the glenoid revealed a significantly larger radius for bone than cartilage, whereas for the humeral head the opposite was the case. Highest ratios for cartilage in the transverse plane were found in the inferior and central areas of the joint surface, whereas the smallest ratios were found in the superior area (Fig.1). The radial mismatch varied between 0.1 mm and 13.6 mm, depending on the measured plane. The results suggest that in abduction, the cartilaginous guidance of the humeral head decreases which might permit the humeral head an anterior-posterior shifting as well as superior-inferior translation.

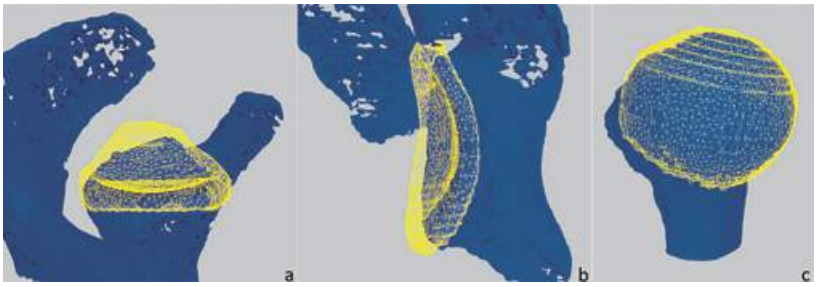

Fig. 1: Visualisation of the cartilaginous (yellow mesh) and osseous structure (blue) in (a) infero-superior view of the glenoid, (b) antero-posterior view of the glenoid and (c) frontal view of the humeral head.

Changes of Density Distribution of the Subchondral Bone Plate after Supramalleolar Osteotomy for Valgus Ankle Osteoarthritis

CT-osteoabsorptiometry (CT-OAM) has been used to visualize subchondral bone plate density distribution regarding to ist mineralization. The purpose of this study was to analyze changes in density distribution of the subchondral bone plate before and after supramalleolar realignment osteotomies due to adaptational processes. We retrospectively analysed pre- and postoperative CT images of patients with post-traumatic unilateral valgus ankle OA by means of CT-OAM. At a mean follow-up of 20 months we observed a significant pre- to postoperative decrease of the mean high-density area ratio in tibia (lateral and posterior area) (p_0.05) and the talus (lateral area) (p_0.05). Pairwise comparison between the pre- and postoperative mineralization at the articular surface showed a significant decrease of the high-density area ratio for the tibia and the talus. The tibial and talar subchondral bone plate density, regarding to its mineralization, decreased after supramalleolar medial closing wedge osteotomy in patients with valgus ankle OA correlating with an improvement of pain symptoms (VAS decreased from 6.2+/-0.9 pre- to 2.8+/- 0.9 postoperatively (p=0.027). The results of this study suggest that realignment surgery may lead to a better load distribution.

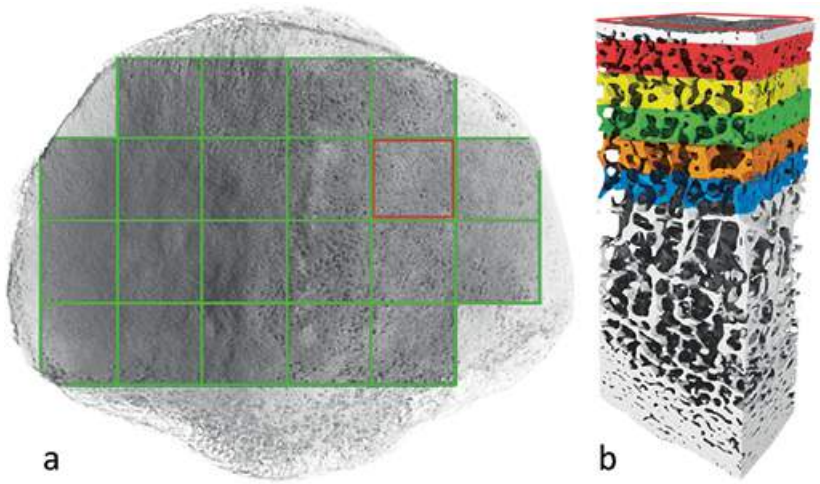

Fig. 2: Method of micro -CT; definition of measurement cube and regions of interest. (A)3D reconstruction, left patella in dorsal view, 21 areas for extraction of measurementcubes marked. (B) Measurement cube with 5 highlighted regions of interest (1st ROI:red; 2nd ROI: yellow; 3rd ROI: green; 4th ROI: orange; 5th ROI: blue) just below the subchondral bone plate.

Insight into the 3D-trabecular architecture of the human patella

The subchondral bone plate (SBP), a dynamic component of the osteochondral unit, shows functional adaptation to long-term loading by distribution of the mineral content in a manner best serving the mechanical demands. Since the received joint-load is transmitted into the trabecular system, the spongy bone should also exhibits topographical differences. To evaluate the regional variations in trabecular architecture, ten physiologic patellae were analysed for defined parameters of bony structure by means of micro-computed tomography (Fig.2). The obtained measurements are: Bone volume fraction (BV/TV); Bone surface density (BS/TV); Trabecular number (Tb.N); Trabecular separation (Tb.Sp); Trabecular thickness (Tb. Th); structure model index (SMI); and the Degree of anisotropy (DA). The evaluated architectural parameter varied within the trabecular system and showed regular distribution patterns (Fig.3). It proved to be distinctive with maxima of material and stability situated below areas of the highest long-term load intake. With increasing depth, the pattern of distribution was persistent but lessened in intensity. The parameters significantly correlated with the density distribution of the SBP. The trabecular network adapts to its mechanical needs and is therefore not homogenously built. Dependent upon the long-term load intake, the trabecular model optimizes the support with significant correlation to the density distribution of the SBP.

Selected Publications

Zumstein V, Kraljevic M, Hoechel S, Conzen A, Nowakowski AM, Müller-Gerbl M. (2014) The glenohumeral joint – a mismatching system? A morphological analysis of the cartilaginous and osseus curvature of the humeral head and the glenoid cavity. J Orthop Surg Res. 13;9:34

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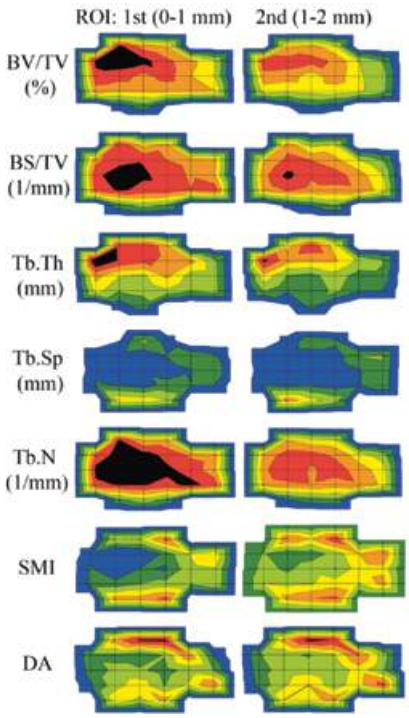

Fig. 3: 2D distribution charts of trabecular architectural parameters for the first two of analysed ROIs (1 and 2 mm below the SBP)

Signal Transduction

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T-cadherin and tissue homeostasis

Cadherins comprise a family of cell-cell adhesion proteins critical to architecture and function of tissues in developing and adult organisms. T-cadherin (T-cad) is peculiar in structure: it lacks transmembrane and cytosolic domains and is membrane-anchored via a GPI moiety, implying distinct functions and molecular circuitry. Analyses based on immunohistochemistry (IHC), gene expression, epigenetic modification and GWAS have ascribed relevance to T-cad in cardiovascular diseases (CVD) and cancer. However biological sense and mechanistic explanations for its functions and/or disease associations are lacking. We focus on delineating T-cad-dependent cellular functions and signal pathway utilization in vascular and cancer cells, with the broader goal being to define basic molecular and biological mechanisms underlying T-cad-mediated control of tissue homeostasis.

T-cadherin in the vasculature

In CVD T-cad protein expressed on vascular smooth muscle cells (SMC) and endothelial cells (EC) is increased. Previous studies addressed T-cad functions and mechanisms of action in EC. Focus over recent years extended to SMC which, through their ability to reversibly transition between contractile/differentiated and synthetic/dedifferentiated phenotypes, crucially determine functionality and structure of vessels and thereby the course of CVD. T-cad upregulation promotes insulin resistance, matrix remodeling and proliferation and reduces contractile capacity, all of which are functional hallmarks of the acquisition of a dedifferentiated phenotype. T-cad directly modulates activity of major signalling checkpoints controlling SMC plasticity *via* Akt/mTORC1 and Akt/GSK3β pathways (Fig. 1). Mechanisms mediating T-cad effects on SMC polarity, migration and cell-matrix adhesion are currently under study. The role of T-cad in autophagy, a process implicated in behavioural flexibility of SMC and important for cell survival during stress, is also under investigation. Overall our research advances knowledge on the role of cadherins in vascular (patho)biology and is relevant for development of therapeutic strategies targeting pathological SMC-driven reparation in CVD, or for harnessing phenotype modulation for tissue regeneration/engineering purposes.

T-cadherin in cancer

T-cad has been implicated in cancer progression primarily on the basis of genetic and epigenetic studies. IHC and functional studies are rare but necessary to understand the role of T-cad in cancer progression. Our work in the context of skin and prostate cancers has shown that T-cad protein and gene expression levels do not always match, highlighting the importance of IHC analysis of expression and cellular distribution in any assessment of disease-associated alterations in T-cad. In normal cutaneous tissue T-cad expression highly depends on cell layer/origin (e. g. squamous and suprabasal low, basal and myoepithelial high, bulge stem and transit-amplifying cells very high) (Fig. 2A). In prostate tissue there is a gradient of T-cad expression across prostate gland epithelial layers (weak in basal, strong in luminal). Expression levels and patterns of T-cad protein in cutaneous and prostate cancers are heterogeneous (increased, decreased and/or absent) and depend on tumor differentiation status and cell-of-origin (Fig. 2B). Together, these studies imply roles for T-cad in cellular differentiation and maintenance of polarized tissue architecture. *In vitro* and *in vivo* functional studies have shown that T-cad influences cancer progression through two distinct mechanisms: by regulating tumor cell differentiation, adhesion, invasion and metastasis and by promoting intratumoral angiogenesis. Signaling effectors are under investigation.

Conclusion

The *"functional predestination"* of T-cad is to control tissue homeostasis through modulation of cellular "differentiation", "guiding" navigation of moving structures, "segregation" of functional tissue compartments and "guarding" integrity of functionally connected tissue layers.

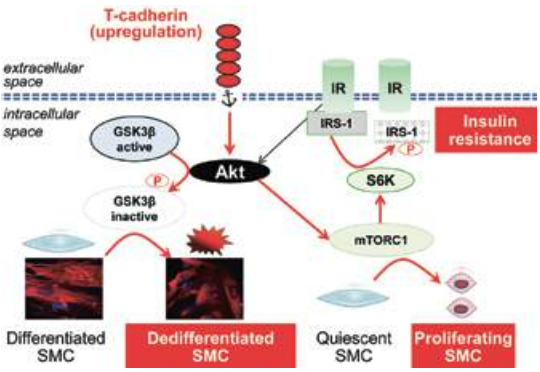

Fig. 1: T-cadherin expression is a determinant of vascular smooth muscle cell (SMC) plasticity and functionality. T-cad is upregulated on SMC during atherosclerosis and restenosis. Functional consequences and involved molecular pathways are depicted.

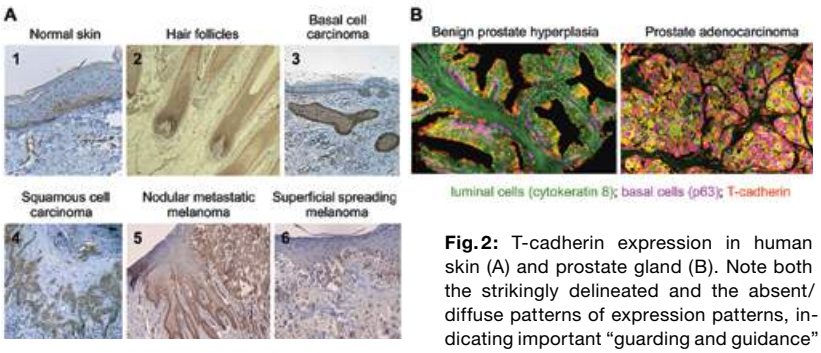

Fig. 2: T-cadherin expression in human skin (A) and prostate gland (B). Note both the strikingly delineated and the absent/diffuse patterns of expression patterns, indicating important "guarding and guidance" roles for T-cadhe-rin in health and disease.

Selected Publications

Frismantiene A, Dasen B, Pfaff D, Erne P, Resink TJ, Philippova M. (2016) T- cadherin promotes vascular smooth muscle cell dedifferentiation via a GSK3beta- inactivation dependent mechanism. Cell Signal 28, 516–530

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Frismantiene A, Pfaff D, Frachet A, Coen M, Joshi MB, Maslova, K, Bochaton-Piallat ML, Erne P, Resink TJ, Philippova, M. (2014) Regulation of contractile signaling and matrix remodeling by T-cadherin in vascular smooth muscle cells: Constitutive and insulin-dependent effects. Cell Signal 26, 1897–1908

Connection to Clinical Practice

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Biomarkers for early detection and risk stratification of cardiovascular disease

Cardiovascular diseases (CVD) based on atherosclerosis remain a chief cause of morbidity and mortality worldwide. We aimed to identify new candidate plasma biomarkers with screening-, diagnostic- or monitoring potential in detecting early atherosclerosis and risk stratification of CVD. We have compiled a wide-ranging clinical data base on a large study cohort (healthy subjects, patients without cardiovascular risk factors, and patients with different stages of atherosclerosis) and a corresponding bank of plasma samples for protein and lipid biomarker analysis. We found that measurement of plasma T-cad relative to levels in healthy subjects has biomarker potential for detecting early atherosclerosis and for evaluating disease severity: an elevation correlates with early, clinically silent atherosclerosis but a decrease correlates with increasing severity of coronary artery disease (CAD) and higher risk for acute coronary syndrome (ACS). We also found opposing correlations between T-cad and its heterophilic ligand adiponectin (APN) in male and female patients, underscoring a need to consider sex as a confounding variable when evaluating biomarker potentials of APN and T-cad. Oxidized phospholipids (OxPL) are key proatherogenic culprits. We developed a novel method for global measurement of the capacity of plasma to inactivate OxPL. A pilot clinical study found a reduced inactivation capacity in hypertension, CAD, ACS and diabetes, indicating potential biomarker value of this parameter in CVD. We also developed a novel LC-MS/MS-based method to identify and quantify OxPL species in plasma and are currently profiling their disease relevance and biomarker potential.

Pulmonary Cell Research

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Linking tissue remodelling and chronic inflammation of the lung

The prevalence of chronic inflammatory lung diseases (CILD) is increasing worldwide since several decades and together they represent the 4th most frequent cause of absence from education and work. Asthma, COPD and fibrotic changes of the lung are the most frequent CILD and are characterised by chronic inflammation and excessive tissue remodelling. The pathologies, however, occur in disease specific patterns and different lung compartments. Recent studies challenged the long held hypothesis that tissue remodelling is caused by chronic inflammation. Today it is hypothesised that inflammation and remodelling occur independently. Furthermore, the likelihood to develop CILD was linked to epigenetic modifications which are imprinted during the last 8 weeks of embryogenesis and early childhood.

Asthma affects 300 million people worldwide and is not curable. The pathogenesis of asthma is not well understood and it is discussed if allergic and chronic asthma are the same disease or if they are 2 diseases which share many pathologies. Since 1922, we have known that a major pathology of asthma is the increase of airway wall thickness which is due to the hypertrophy and hyperplasia of airway smooth muscle cells (ASMC). Recent studies in asthma patients showed that reduction of ASMC significantly reduced symptoms. ASMC are the major cause of airway constriction, immune cell infiltration, and of chronic inflammation. Together with our colleagues we search for novel targets aiming to develop curative therapies. Recently we described a novel signalling pathway which is constitutively activated in sub-epithelial cells of asthma patients and leads to the secretion of pro-inflammatory cytokines, increased proliferation and deposition of extracellular matrix components. This signalling pathway involves Erk1/2, Stat1 and PRMT1 and is activated by all tested asthma triggers (IgE, TGF-β, PDGF-BB, IL-4, TNF-α). Interestingly, the regulation of this pathway occurs on the epigenetic level and we linked this to our earlier finding: the lack of C/EBP-α expression in asthmatic ASMC. In regard to new therapies, the inhibition of PRMT1 may be easier to achieve than the expression of C/EBP-α.

COPD is the 3rd most frequent cause of death in the U.S.A. and ranked 4th in Switzerland; thus, COPD caused more death than diabetes or neuro-degenerative diseases. The susceptibility to develop COPD seems to be defined by epigenetic imprinting during embryo development and later exposure to the known triggers which include cigarette smoke, fine ash, industrial fine dust and organic inhaled matters. Cigarette smoke is the main trigger of COPD which affects 20% of all smokers. COPD is deadly and cannot be cured with any available drugs. In early stages, COPD shows similar tissue remodelling pathologies as asthma, however, in small bronchi. In late stages, COPD is characterised by tissue degradation (emphysema). Clinical data showed that combining long acting beta2-agonists with long acting muscarinic receptor antagonists achieved better symptoms control and improved lung function compared to other treatments. Using our human cell model with COPD cells, we provided evidence that this drug combination has beneficial effects on extracellular matrix remodelling, thus providing the molecular biological rational of clinical studies.

Idiopathic pulmonary fibrosis (IPF) is a rare but devastation non-malignant lung disease with unknown cause and limited therapeutic options. We have recently shown that fibrotic remodelling can be controlled by the two novel drugs, nintedanib and pirfenidone, however, clinical studies have demonstrated only limited therapeutic benefit for IPF patients. In collaboration with the University of Bern, we obtained data showing that human fibrotic lung tissue contains increased numbers of pluripotent cells which show characteristics of stem cells. *In vitro* studies demonstrated that these pluripotent stem cells exhibit anti-fibrotic effects via fibroblasts and epithelial cells. Our current studies aim to understand the role of these stem cells in tissue fibrosis and to elucidate the mechanism of their anti-fibrotic properties.

Selected Publications

Hostettler KE, Zhong J, Papakonstantinou E, Karakiulakis G, Tamm M, Seidel P, Sun Q, Mandal J, Lardinois D, Lambers C, Roth M. (2014) Anti-fibrotic effects of nintedanib in lung fibroblasts derived from patients with idiopathic pulmonary fibrosis. Respiratory Research 15,157

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Sun Q, Liu L, Mandal J, Molino A, Stolz D, Tamm M, Lu S, Roth M. (2016) PDGF-BB Induces PRMT1 Expression through ERK1 /2 Dependent STAT1 Activation and Regulates Remodeling in Primary Human Lung Fibroblasts. Cellular Signalling 28,307–15

Seidel P, Costa L, Sun Q, Lardinois D, Tamm M, Roth M (2016). The MNK-1/eIF4E pathway as a new therapeutic pathway to target inflammation and remodelling in asthma. Cellular Signalling 28,1555–62

Sun Q, Li L, Wang H, Mandal J, Khan P, Hostettler KE, Stolz D, Tamm M, Molino A, Lardinois D, Lu S, Roth M. (2016) Constitutive high expression of PRMT1in asthmatic airway smooth muscle cells is caused by reduced MicroRna-19a and leads to enhanced remodelling. Journal of Allergy and Clinical Immunology in press

Connection to Clinical Practice

Translational medicine studies in chronic inflammatory lung diseases

Our studies are focused on chronic inflammatory lung diseases. We link basic and clinical research to find novel bio-markers and therapeutic targets for asthma, COPD, ACOS, and idiopathic fibrosis. None of these diseases is easy to diagnose, and despite symptom control, curative therapies are not available. All studies are based on large patient cohorts, diagnosed according to international guidelines. We preform risk factor analysis and validate new biomarkers, as predictors for exacerbation and survival of patients.

The clinical studies are enabled by a close collaboration of pulmonologists, thoracic surgeons, haematologists, pathologists, and basic researchers. This translational approach benefits from the close co-location of the Clinic of Pneumology and the research laboratory (DBM). Using human lung samples of patients we are able to isolate primary diseased human epithelial cells, fibroblasts and bronchial smooth muscle cells and provide them to our collaborators in other countries. The cells are analysed for disease specific expression and regulation patterns of biomarkers and inflammatory mediators. This also allows us to identify novel disease specific pathophysiological pathways and test new medications on the cellular level. Components of tissue remodelling are studied under the influence of allergic and non-allergic stimuli. In addition to this translational research projects numerous investigator driven non-commercial randomised studies are performed to optimise patient's safety during bronchoscopy in COPD patients. Our clinic leads collaborative studies with groups in Germany, Italy, Spain, France, the Netherlands, Belgium, UK, Serbia and Greece.

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Molecular pathogenesis of myeloproliferative neoplasms

Myeloproliferative neoplasms (MPN) are a group of blood diseases characterized by aberrant proliferation of precursors of the myeloid, erythroid and megakaryocytic lineages. They represent clonal stem cell disorders with a tendency towards leukemic transformation. Currently, no curative therapy is available. MPNs comprise 3 entities: polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF). The goal of our studies is to advance the understanding of the molecular events that initiate MPN and influence its progression to leukemia. A recurrent mutation Janus kinase 2 (JAK2) gene that substitutes a valine to phenylalanine at position 617 (JAK2-V617F) is present in a majority of patients with MPN, in particular PV. This mutation leads to activation of the Jak2 tyrosine kinase and represents a driver for the proliferation of hematopoietic cells. Activating mutations calreticulin (CALR) and the thrombopoietin receptor (MPL) represent other frequent driver events. Despite this progress, several questions remain unsolved including how a single JAK2 mutation causes three different MPN phenotypes, what other genes might be involved and what determines the progression to acute leukemia. We are examining these questions by combining three approaches: molecular studies in patients with sporadic MPN, genetic analysis of familial MPN and transgenic mouse models that mimic the human disease.

Analysis of clonal progression in MPN

Using next generation sequencing, we determined the mutational profiles of MPN patients and analyzed correlations with clinical outcome and prognosis. We found that increasing number of somatic mutations per patient was associated with higher risk of transformation to acute leukemia and with reduced survival (Fig. 1). Some gene mutations, e.g. TP53 had a particularly bad prognostic impact. By examining individual colonies grown from patient's peripheral blood, the clonal architecture can be precisely determined. In a subset of patients with sporadic MPN additional somatic mutations can either precede or occur after the acquisition of JAK2-V617F. We are examining the impact of the clonal architecture on outcome and response to therapy.

Familial predisposition for MPN

Familial syndromes resembling MPN can be grouped into two classes:

1. Inherited disorders with high penetrance and polyclonal hematopoiesis.
2. Hereditary predisposition to true MPN, with low penetrance, clonal hematopoiesis and occurrence of somatic mutations, e.g. in JAK2-V617F.

We identified mutations in the thrombopoietin (THPO) gene as the cause for an inherited form of thrombocythemia in several families with a "class 1" phenotype. In another family we found a previously described mutation in MPL. However, in the majority of families neither THPO nor MPL is mutated. The search for these disease genes is ongoing. Families with "class 2" phenotype are more common than generally assumed. These germ line mutations increase the likelihood of acquiring a somatic JAK2-V617F mutation. We are using genetic methods to map the locus for these pre-disposing mutations.

Mouse models for MPN

We generated JAK2-V617F transgenic mice that express the human JAK2-V617F. This conditional construct can be activated by Cre-recombinase. Depending on the mode of Cre-mediated activation, these mice developed a phenotype resembling ET with strongly elevated platelet counts or a PV-like phenotype with increased hemoglobin, thrombocytosis and neutrophilia. A major focus of our research is to examine the nature of the MPN initiating stem cells and their interactions with the

bone marrow microenvironment. We demonstrated that MPN can be initiated by transplanting single hematopoietic stem cells that carry JAK2-V617F as the sole genetic alteration and that loss of function mutations in Ezh2 promoted disease initiation from single cells and accelerated disease towards myelofibosis. We are dissecting the contributions of additional genes to the pathogenesis using conditional knockout models. We are also using our mouse models for pre-clinical screening of Jak2 inhibitors and other potential therapeutic agents.

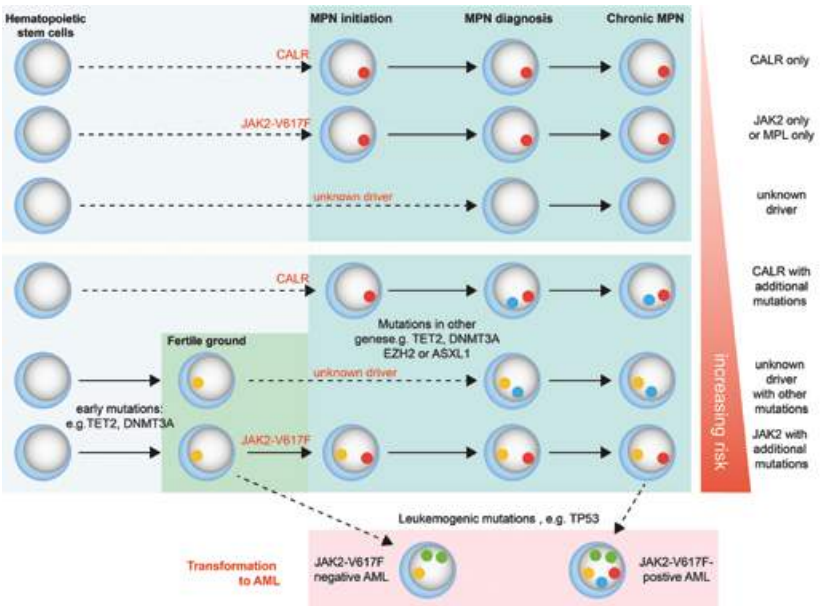

Fig. 1: Model of MPN disease evolution and risk stratification in correlation to mutational events.

Selected Publications

Shimizu T, Kubovcakova L, Nienhold R, Zmajkovic J, Meyer SC, Hao-Shen H, Geier F, Dirnhofer S, Guglielmelli P, Vannucchi AM, Feenstra JD, Kralovics R, Orkin SH and Skoda RC. (2016) Loss of Ezh2 synergizes with JAK2-V617F in initiating myeloproliferative neoplasms and promoting myelofibrosis. The Journal of Experimental Medicine 213, 1479–1496

Grisouard J, Li S, Kubovcakova L, Rao TN, Meyer SC, Lundberg P, Hao-Shen H, Romanet V, Murakami M, Radimerski T, Dirnhofer S and Skoda RC. (2016) JAK2 exon 12 mutant mice display isolated erythrocytosis and changes in iron metabolism favoring increased erythropoiesis. Blood 128, 839–851

Grisouard J, Shimizu T, Duek A, Kubovcakova L, Hao-Shen H, Dirnhofer S and Skoda RC. (2015) Deletion of Stat3 in hematopoietic cells enhances thrombocytosis and shortens survival in a JAK2-V617F mouse model of MPN. Blood 125, 2131–2140

Lundberg P, Takizawa H, Kubovcakova L, Guo G, Hao-Shen H, Dirnhofer S, Orkin SH, Manz MG and Skoda RC. (2014) Myeloproliferative neoplasms can be initiated from a single hematopoietic stem cell expressing JAK2-V617F. The Journal of Experimental Medicine 211, 2213–2230

Lundberg P, Karow A, Nienhold R, Looser R, Hao-Shen H, Nissen I, Girsberger S, Lehmann T, Passweg J, Stern M, Beisel C, Kralovics R and Skoda RC. (2014) Clonal evolution and clinical correlates of somatic mutations in myeloproliferative neoplasms. Blood 123, 2220–2228

Duek A, Lundberg P, Shimizu T, Grisouard J, Karow A, Kubovcakova L, Hao-Shen H, Dirnhofer S and Skoda RC. (2014) Loss of Stat1 decreases megakaryopoiesis and favors erythropoiesis in a JAK2-V617F-driven mouse model of MPNs. Blood 123, 3943–3950

Connection to Clinical Practice

Prof. Jakob Passweg and Dr. Pontus Lundberg
Division of Hematoloy, University Hospital Basel

Improved diagnostics of MPN and new therapeutic approaches: From bench to bedside

The detection of somatic driver gene mutations in JAK2, CALR and MPL is now a key step in the diagnostic approach to MPN in accordance with the revised WHO criteria. Furthermore, mutations in genes that can modify the course of the disease, such as ASXL1, EZH2, or TP53 are now in the focus interest and are likely to become increasingly important in clinical decision making. To cover the increasing need for the comprehensive molecular diagnostics of MPN, we developed a next generation sequencing (NGS) based gene panel that allows a molecular classification of patients with myeloid neoplasm. This panel is based on our NGS study (Lundberg et al, Blood 2014), and is now being used for the routine diagnostics of MPN patients.

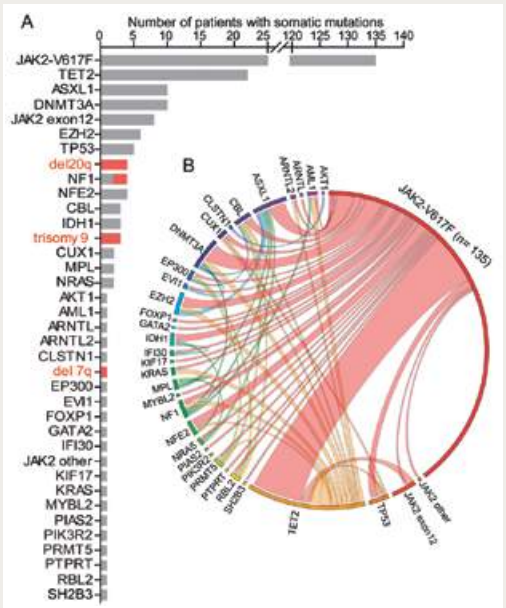

Fig. 2: Frequency and distribution of mutations in patients with MPN. (A) Number of patients with mutations in the genes as indicated. Red bars and red text indicate chromosomal aberrations. (B) Circos plot illustrating co-occurrence of somatic mutations in the same individual. The length of the arc corresponds to the frequency of the mutation, while the width of the ribbon corresponds to the relative frequency of co-occurrence of two mutations in the same patient.

Embryology and Stem Cell Biology

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Regulation of neural stem cell fate

Deciphering the mechanisms of neurogenesis
Development of the brain is controlled in a precise and organized fashion, but the mechanism controlling the differentiation of neuron-types in the cerebral cortex are unclear. The adult mammalian brain contains neural stem cells (NSCs) that continue to generate neurons in defined regions and contribute to brain homeostasis and function. Our understanding of the control of NSC activity and maintenance is rudimentary, but these processes have implications for brain function and cancers including gliomas. Using mouse genetics *in vivo* and in cell culture we are trying to understand the molecular mechanisms controlling NSC activity and fate during development, adulthood and gliomas formation.

Systems biology of forebrain development
As part of the SystemX.ch project NeuroStemX, we analyze neurogenesis during cortical development. NSCs of the developing cerebral cortex generate six layers of cortical neurons in a precise inside-out temporal fashion. The neurons within each layer are functionally distinct, express specific markers and transcriptional regulators, and are born at precise times during development. The number of neurons within each layer is precisely controlled through sequentially modulation of NSC and progenitor cell fate. The mechanisms orchestrating neural progenitor fate in the developing cerebral cortex are not understood. We have addressed, at the systems biology level, whether all NSCs in the developing cortex have the same potential and respond to the same fate cues to contribute to all neuronal cell-types. We generated high-resolution transcriptome analyses of NSCs, progenitors and newborn neurons by next generation RNA sequencing at the population and single cell levels at each day of cortical development. We are using computational modeling of signaling and transcriptional regulatory networks to uncover the complex networks and switches in signaling that control NSC fate decisions.

Regulation of adult NSC fate by Notch and Drosha
Multi-lineage potential is an adult NSC trait. We showed that the RNAseIII Drosha is an intrinsic regulator of adult NSC maintenance and differentiation. Drosha silences NFIB in NSCs by cleaving hairpins in its mRNA thereby repressing expression. Our findings revealed a novel mechanism for the maintenance and oligodendrocyte fate restriction of adult NSCs. We continue to study targets of Drosha in the NSCs in embryonic and adult NSCs. Neurogenesis continues in adult forebrain from quiescent NSCs. To generate neurons, NSCs activate and enter cell cycle. Notch signaling is critical in this process and we found that Notch2 signaling conveys quiescence to adult NSCs by repressing cell cycle genes and neurogenesis. Although neurogenesis occurs at all location of the developing embryonic brain, in adults, neuron production is restricted to specific brain regions. We identified dormant adult NSCs in niches outside the classical neurogenic zones. These NSCs are regulated by Notch2 signaling and retain neurogenic potential responding to pathophysiological stimuli to control mouse behavior. Thus, we identified novel NSCs in the brain that are held in a reversible, inactive state.

Notch in forebrain tumor subtypes
Notch signaling maintains NSC and as been proposed to be oncogenic. However, inactivating mutations in Notch receptors suggest that Notch signaling has tumor suppressor functions in human gliomas. We generated genetic mouse models that simulate different human glioma subtypes. These models enable us to study Notch function in brain tumor formation and growth. We identified a tumor suppressor function for Notch in forebrain tumor subtypes. Inactivating mutations in the Notch1 and Notch2 receptor genes accelerates growth of some gliomas. Con-

versely, activation of the Notch pathway reduces glioma growth. We could confirm these findings in human glioma data finding that high Notch activity correlates with distinct glioma subtypes, increased patient survival, and lower tumor grade. We are studying the role of Notch in other novel brain tumor models to understand how Notch signaling could be used as a therapeutic target for gliomas.

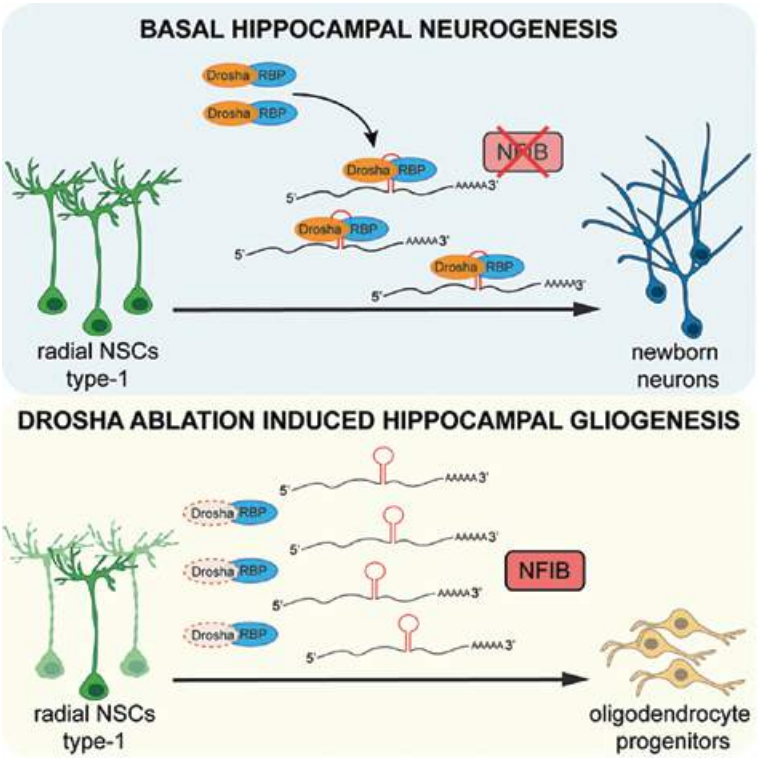

Fig. 1: The RNAse Drosha is a key component of the microRNA microprocessor but also regulates cell fate by directly destabilizing mRNAs of fate determining factors. Drosha is known for its cleavage of pri-miRNA transcripts to generate pre-miRNAs, the precursors of mature microRNAs. Drosha also processes hairpin structures in mRNAs of the proneural transcription factors Ngn2, NeuroD1 and NeuroD6 in NSCs during development (Knuckles *et al.* 2012). In the adult brain, NSCs generate neurons, astrocytes and oligodendrocytes, except in the hippocampus where oligodendrocytic differentiation is blocked. We have recently shown that NSC maintenance in the hippocampus, and their bias against oligodendrocytes differentiation, is controlled by repression of NFIB expression. Under normal conditions (basal hippocampal neurogenesis), hippocampal NSCs do not express NFIB protein as its mRNA is rapidly degraded. We showed that Drosha is responsible for this degradation of NFIB mRNA by cleaving specific hairpin structures formed by the transcript. This intrinsic regulation of NFIB expression enables NSCs to remain and self-renew but also prevents them from differentiating into oligodendrocytes. If Drosha is inhibited or blocked, NFIB mRNA stabilizes, the transcript factor is expressed and hippocampal NSCs erroneously differentiate into oligodendrocytes (Drosha ablation induced hippocampal gliogenesis) (Rolando *et al.* 2016).

Selected Publications

Rolando C, Erni A, Grison A, Beattie R, Engler A, Gokhale PJ, Milo M, Wegleiter T, Jessberger S and Taylor V. (2016) Multipotency of Adult Hippocampal NSCs *In Vivo* Is Restricted by Drosha/NFIB. Cell Stem Cell *19,* 653–662

Giachino C, Boulay JL, Ivanek R, Alvarado A, Tostado C, Lugert S, Tchorz J, Coban M, Mariani L, Bettler B, *et al.* (2015) A Tumor Suppressor Function for Notch Signaling in Forebrain Tumor Subtypes. Cancer Cell *28*, 730–742

Giachino C, Barz M, Tchorz JS, Tome M, Gassmann M, Bischofberger J, Bettler B and Taylor V. (2014a) GABA suppresses neurogenesis in the adult hippocampus through GABAB receptors. Development *141,* 83–90

Giachino C, Basak O, Lugert S, Knuckles P, Obernier K, Fiorelli R, Frank S, Raineteau O, Alvarez-Buylla A and Taylor V. (2014b) Molecular diversity subdivides the adult forebrain neural stem cell population. Stem Cells *32,* 70–84

Rolando C and Taylor V. (2014) Neural stem cell of the hippocampus: development, physiology regulation, and dysfunction in disease. Curr Top Dev Biol *107,* 183–206

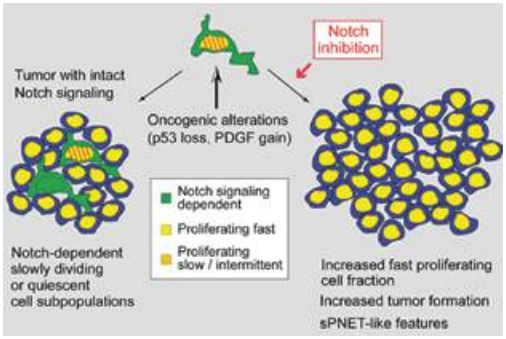

Fig. 2: Gliomas are some of the most devastating cancers and the prognosis for patients with high-grade gliomas is very bad with only ineffective and highly aggressive treatments being available. Notch signaling has been implicated as being oncogenic and inducing gliomas, particularly in the most aggressive forms of the disease, glioblastoma multiforma. We generated murine models of human gliomas that lack the tumor suppressor p53 and express the growth factor PDGF. We assessed the role of Notch signaling in these tumors by genetic ablation and over expression. We could show that these model glioblastomas contain cells where Notch is active, however, in contrast to expectations, deleting Notch signaling increases tumor growth indicating that Notch signaling, in this form of brain tumor, functions as a tumor suppressor. We were able to support these findings by analyzing human tumor data that also indicated that in some human gliomas sub-types, Notch signaling likely functions as a tumor suppressor (Giachino *et al.* 2015). These results open up important new directions for potential therapies for patients with gliomas.

Developmental Genetics

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Signal Integration by Gene Regulatory Landscapes during Mouse Limb Bud Organogenesis – Robustness and Evolutionary Diversification

We are interested in understanding the molecular mechanisms underlying the regulatory circuits that govern vertebrate organogenesis. To this aim we study how gene regulatory landscapes integrate multiple signaling inputs into robust but highly dynamic transcriptional outputs. We are taking advantage of our in-depth knowledge of the self-regulatory signaling systems that control vertebrate limb bud organogenesis. In particular, we are identifying the functionally relevant *cis*/*trans*-regulatory interactions that control the establishment and propagation of this self-regulatory signaling system during initiation and progression of mouse limb bud organogenesis. Often, gene expression is regulated by multiple *cis*-regulatory modules (CRMs) that are scattered in large genomic landscapes, but how these landscapes integrate transcriptional inputs is still largely unknown. Therefore, we profile chromatin architecture, epigenetic marks, interaction of transcriptional regulators with CRMs and transcriptomes to identify the relevant CRMs, which are functionally analysed by CRISPR/Cas9 genome editing (Fig. 1). Experimental analysis is combined with bioinformatics and *in silico* simulations to reveal gene regulatory networks (GRNs) and interaction kinetics. A key node in the self-regulatory limb bud signaling system is the BMP antagonist *Grem1,* whose expression is controlled by a large genomic landscape integrating inputs from all major signaling pathways. Within the *Grem1* landscape, we have identified the region required for limb bud expression, which encodes three CRMs regulated by key transcriptional regulators in limb buds. Currently, we are studying these CRMs that appear to regulate *Grem1* expression in a cooperative manner, possibly as part of a super enhancer. As modern tetrapod limbs display amazing evolutionary diversity, a second fascinating aspect of your research are insights into how this robust and self-regulatory signaling system evolved and diversified. Recently, we

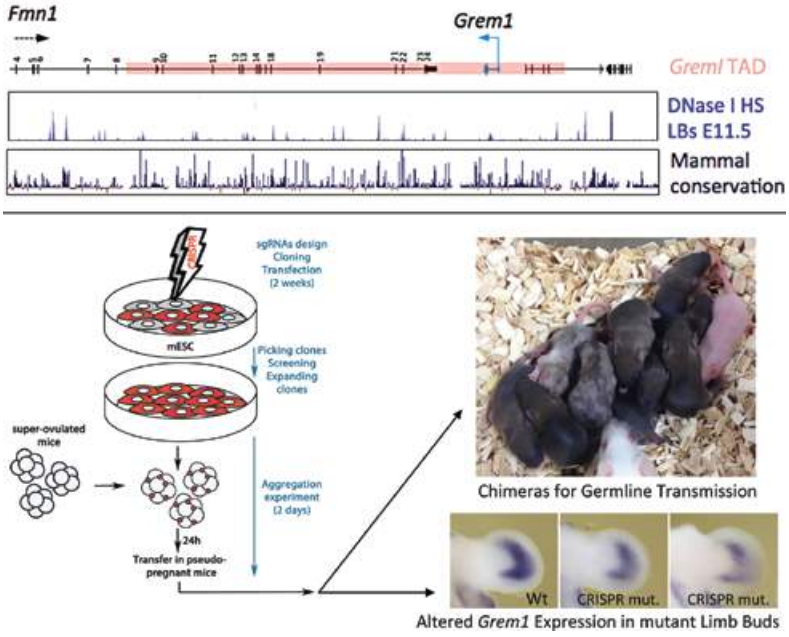

Fig. 1: CRISPR/Cas9-based functional analysis of candidate CRMs in genomic landscapes

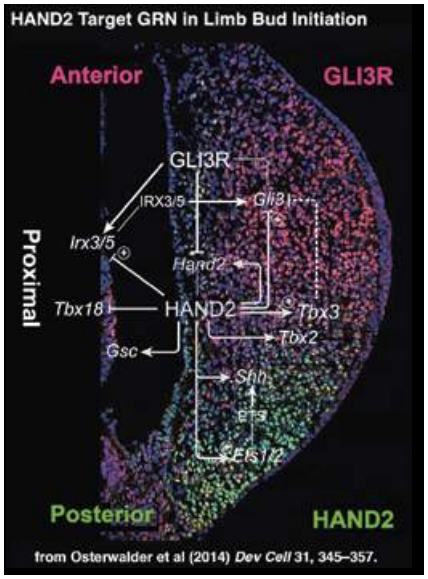

Fig. 2

showed that one of the molecular alterations underlying evolution of the streamlined limb skeletons characteristic of artiodactyls is the inability of limb bud mesenchymal cells to sense the morphogenetic SHH signal. This is due to functional degeneration of a CRM controlling up-regulation of the SHH receptor *Ptch1* in the limb bud mesenchyme.

Another key process is the establishment of the signaling centers controlling proliferation and patterning of progenitor cells. In limb buds, the SHH signaling center is established in the nascent mesenchyme as the limb bud forms. We have shown that the HAND2 transcription factor is essential to this process and polarization of the nascent mesenchyme. ChIP-Seq in combination with genetic analysis revealed the gene regulatory logic by which the HAND2-target GRN establishes proximal, anterior and posterior compartments and activates *Shh* expression in early limb buds (Fig. 2). Recently, we have shown that HAND2 controls similar GRNs during early heart development. Our studies begin to reveal the similarities in the HAND2-target GRNs controlling both limb and heart organogenesis, which points to a possible shared origin of these two structures.

Finally, we have shown that SMAD4, which is part of the transcriptional complexes mediating response to canonical BMP signaling, is essential for normal limb bud development. We have used ChIP-Seq and comparative transcriptome analysis to identify the genes and GRNs that are regulated by SMAD4 chromatin complexes. Our analysis provides insight into the functionally relevant cis-regulatory dynamics of SMAD4-mediated BMP signal transduction during limb bud organogenesis and chondrogenic differentiation. With respect to the latter, we are performing an in depth analysis of the functions of the BMP antagonists Grem1 and *Noggin* and SMAD4-mediated BMP/TGFß signal transduction during chondrogenesis and digit ray formation. Understanding BMP functions in the switch from proliferating mesenchymal to differentiating chondrogenic progenitors is directly relevant to development-inspired engineering of cartilage (joint SNF project with I. Martin).

Selected Publications

Zuniga A. (2015) Next generation limb development and evolution: old questions, new perspectives. Development 142, 3810–3820

Vaillant C, Valdivieso P, Nuciforo S, Kool M, Schwarzentruber-Schauerte A, Méreau H, Cabuy E, Lobrinus JA, Pfister S, Zuniga A, Frank S, Zeller R. (2015) Serpine2/PN-1 is required for Proliferative Expansion of Pre-Neoplastic Lesions and Malignant Progression to Medulloblastoma. PLoS One 10, e0124870 doi: 10.1371/journal.pone.0124870

Van Dusen NJ, Casanovas J, Vincentz JW, Firulli BA, Osterwalder M, Lopez-Rios J, Zeller R , Zhou B, Grego-Bessa J, De La Pompa JL, Shou W, Firulli AB. (2014) Hand2 is an Essential Regulator for Two Notch-Dependent Functions within the Embryonic Endocardium. Cell Reports 9 , 2071–2083

Osterwalder M, Speziale D, Shoukry M, Mohan R, Ivanek R, Kohler M, Beisel C, Wen X, Scales SJ, Christoffels VM, Visel A, Lopez-Rios J, Zeller R. (2014) HAND2 Targets Define a Network of Transcriptional Regulators that Compartmentalize the Early Limb Bud Mesenchyme. Dev Cell 31, 345–357

Lopez-Rios J, Duchesne A, Speziale D, Andrey G, Peterson KA, Germann P, Ünal E, Liu J, Floriot S, Barbey S, Gallard Y, Müller-Gerbl M, Courtney AD, Klopp C, Rodriguez S, Ivanek R, Beisel C, Wicking C, Iber D, Robert B, McMahon AP, Duboule D, Zeller R. (2014) Attenuated sensing of SHH by Ptch1 underlies evolution of bovine limbs. Nature 511 , 46–51. (Article)

Connection to Clinical Practice

Prof. Ivan Martin
DBM and USB

Development – inspired engineering of cartilage

(Joint project Zeller/Martin groups)

Mesenchymal stromal/stem cells (MSC) are give rise to various tissue types under lineage specific differentiation conditions, which includes cartilage, the main focus of our research. Using MSCs for generating cartilage remains challenging due to (1) the heterogenous nature of MSC populations, which likely only contain a small and variable fraction of “stem” cells and/or early progenitors; (2) their compromised potency after *in vitro* expansion and (3) inefficient cartilage differentiation. We have done an indepth characterization of a rare population of adult mouse endosteum derived MSC-type cells as Sca-1+ PDGFR-α+ population (PαS; previously described by Matsuzaki an co-workers) as these cells posses a very robust tri-lineage differentiation potential. We have been able to characterize four subpopulations of PαS cells by FACS analysis. In particular, ontogenic analysis revealed the progressive appearance of the four PαS subpopulations during mouse embryonic limb development. A development-inspired culture protocol was used to generate stable cartilage templates from the PαS subpopulations and their endochondral bone forming potential was assessed by engraftment of cartilage constructs into nude mice. This analysis revealed the distinct potential of one of the PαS subpopulations for endochondral bone formation and support of host-derived hematopoiesis. We have initiated transcriptome analysis to enable in depth characterisation of these four PαS subpopulations.

This research is funded by an SNF grant (main applicant: I. Martin, co-applicant: R. Zeller)

Oncology

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The major goal of this focal area is to support and expand research in the field of molecular and clinical oncology in Basel. In particular, we aim at bridging the gaps between basic, translational, and clinical oncology research ongoing at the University of Basel and the biotech and pharmaceutical industry in the Basel area. Ultimately, the focal area should enforce collaborative efforts and common projects between various research groups, research institutes and pharmaceutical industry and between different disciplines. An added value is seen in innovative projects that eventually pay off by being transferred to a clinical setting. This research program relies critically on the participating individuals' enthusiasm and initiatives.

The Focal Area Oncology is currently chaired by Professor Gerhard Christofori, leader of the "Tumor Biology" group at the DBM, and by Professor Christoph Rochlitz, head of Clinical Oncology at the University Hospital. The program focuses on two major topics: first to support basic, translational, and clinical research by generating opportunities for oncology research, for example by hosting new recruitments within the DBM. In 2015, we were happy to welcome Prof. Nicola Aceto as SNF-Assistant Professor to lead the research group "Cancer Metastasis" and, in 2016, Prof. Mohamed Bentires-Alj as Professor of Experimental Surgical Oncology to lead the research group "Tumor Heterogeneity, Metastasis and Resistance". Both are now highly active in shaping the Focal Area Oncology with new initiatives and contributions.

The second focus of the Focal Area Oncology is to increase communication between the various researchers, clinicians and pharmaceutical company representatives in Basel and to foster scientific exchange and technological collaboration, also with the other Focal Areas of the DBM. Towards this goal, one-day symposia are organized to offer platforms for the discussion of research progress and for the exchange of ideas. Many members of the DBM Oncology Program are also engaged in the Basel Signaling Alliance, a center of excellence at the University of Basel, and in 2014 they have organized another high-impact international conference in Basel,"Kinome III". A more recent new addition to the Focal Area Oncology is the Basel Breast Consortium (BBC) which integrates basic, translational and clinical research on breast cancer and has already been highly successful in organizing progress report meetings and annual conferences. In addition, renowned international cancer researchers are invited to present lectures within the DBM Oncology Program Seminars, and impromptu Guest Seminars complete the seminar activities of the research program. Many members of the Foal Area Oncology are also actively participating in the European Cancer Center (EuCC), an oncology platform initiative of the Universities of Freiburg, Germany, Strasbourg, France, and Basel. Finally, specific advanced courses in experimental cancer research and in cell signaling are offered to Master and PhD students. Thus far, these communication activities have resulted into highly successful collaborations and research networks, manifested also by the accomplishments of several SystemsX.ch project grants, SNF Sinergia grants, and a highly prestigious European Research Council (ERC) Synergy grant. Of note, a recent highly successful biotech startup of the University of Basel (Piqur Therapeutics AG) also has major roots in the DBM Oncology Program. Accordingly, many of these efforts are part of international and national research initiatives that cover innovative approaches to can-

cer research and treatment, including research on cancer genetics and genetic instability, cancer epigenetics, angiogenesis and metastasis, signal transduction, cancer stem cells, tumor immunology and immunotherapy, tumor imaging and novel therapeutic regimen. In the years to come, we still need to enforce scientific exchange between basic and patient-oriented research in order to facilitate the identification of clinical problems for the design of appropriate and innovative basic research approaches and, on the other hand, to further improve on the rapid translation of basic research results into clinical application.

Cancer Metastasis

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Analysis of circulating tumor cells to dissect the biology of human cancer metastasis

More than 90% of cancer-related deaths, corresponding to more than eight million people worldwide each year, are due to the development of a metastatic disease. Clearly, these numbers reflect our limited understanding of the key processes that drive human cancer metastasis, and the need to develop new therapies that suppress the spread of cancer.

Several unsolved questions frame the metastasis research field, including the search of those molecular events that are fundamental for the metastatic process, and that would represent exceptional therapeutic targets. Cancer cells that leave the primary tumor site and are transported through the circulation to distant organs are referred to as circulating tumor cells (CTCs). CTCs are used as a noninvasive source of cancer cells for analysis of tumor genotypes (i.e. so-called liquid biopsy), yet their characterization is also an exceptional opportunity to dissect the biology of blood-borne metastasis. While CTCs are extraordinarily rare in circulation, even in patients with metastatic cancer (approximately one cancer cell among a billion normal blood cells), their isolation is highly dependent upon technological constraints. However, remarkable advances in the microfluidics field have now enabled the isolation of viable CTCs from virtually all cancer types, revealing highly unexpected features of the metastatic process.

For example, while the majority of CTCs circulate as single cells, they can also be found as clusters of 2–50 cells (a.k.a. CTC-clusters), with the ratio of single *versus* clustered CTCs varying significantly among different patients, and along disease progression. While CTC-clusters have been previously observed in human specimens, their role in the metastatic process was unknown. When combining microfluidic technologies for CTC isolation, single cell resolution RNA sequencing, patient samples and mouse models, we recently demonstrated that CTC-clusters represent key players in the metastatic process. First, we understood that the presence of CTC-clusters in the bloodstream of patients with breast and prostate cancer is associated with a shorter metastasis-free survival and overall survival, respectively, compared to patients in whom only single CTCs are found. Second, adopting multicolor mouse models to trace metastatic cancer cells *in vivo*, we concluded that CTC-clusters are oligoclonal units derived from the primary tumor (as opposed to be derived from intravascular aggregation events or the progeny of a single CTC), and that they are up to 50-fold more metastatic than single CTCs. Third, with a single cell-resolution RNA sequencing approach applied to human CTC-clusters and matched single CTCs from individual patients, we identified the cell-cell junction component plakoglobin to be required for CTC-clustering and metastasis. Together, these results highlight CTC-clusters as a previously unappreciated, yet potentially targetable mechanism of cancer dissemination.

Our research is now focused on the identification of the key vulnerabilities of CTC-clusters. In collaboration with Prof. Christoph Rochlitz, Prof. Viola Heinzelmann Prof. Alfred Zippelius and Prof. Walter Weber at the University Hospital Basel we routinely isolate CTCs from the blood of patients with metastatic cancers. In the lab, we apply microfluidics technology to human and mouse blood specimens, and adopt next-generation sequencing, molecular and computational biology, CTC cultures as well as loss of function screenings in xenograft models. Together, our approach aims to gain fundamental insights into the biology of CTC-clusters, and to identify novel therapeutic targets to suppress the metastatic spread of cancer.

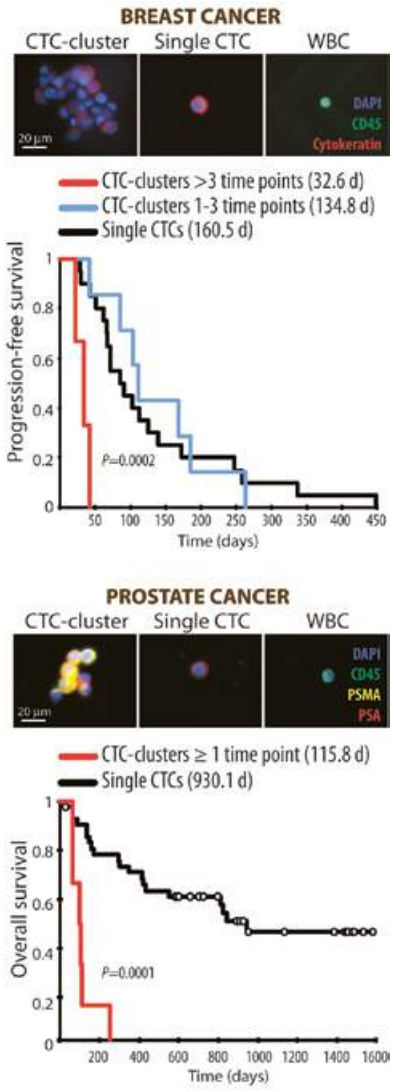


Fig. 1: The presence of CTC-clusters in patients with cancer correlates with poor prognosis. Kaplan-Meier analysis of patient data showing that the presence of CTC-clusters correlates with reduced progression-free survival and overall survival in patients with breast *(top)* and prostate cancer *(bottom)*, respectively.

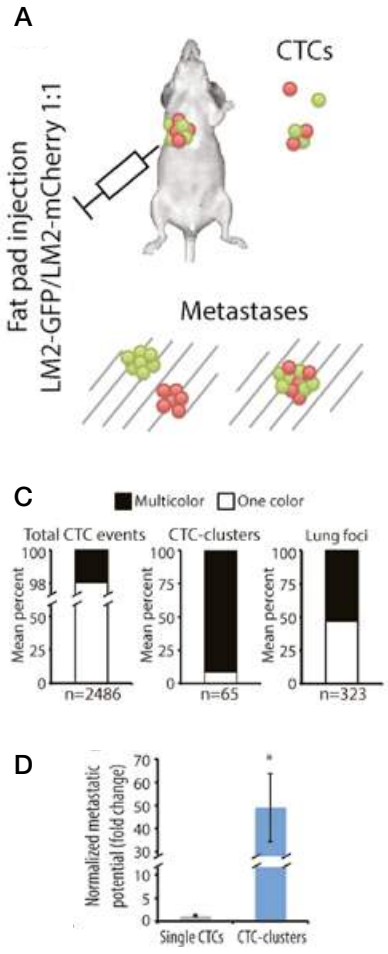
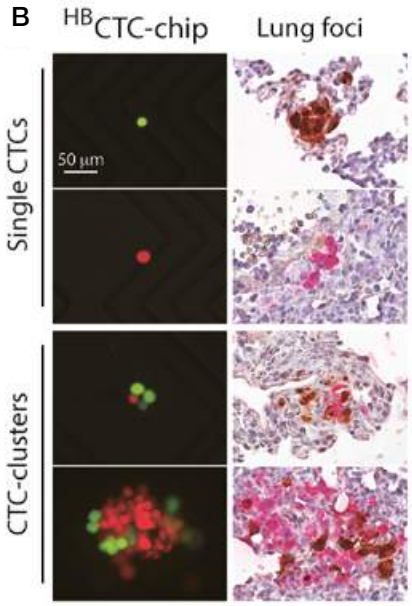


Fig. 2: CTC-clusters as metastatic precursors. **A)** Schematic of the experiment leading to spontaneous formation of multicolor CTC-clusters vs monocolor single CTCs from a primary breast tumor. **B)** Immunofluorescence images of CTCs *(left)* and immunohistochemistry staining of metastatic foci *(right)*. **C)** Bar graphs showing that the vast majority of CTC-clusters is multicolor and gives rise to multicolor metastatic foci. **D)** Bar graphs showing that CTC-clusters are up to 50-fold more metastatic than single CTCs.



Connection to Clinical Practice

Prof. Dr. Christoph Rochlitz, Prof. Dr. Viola Heinzelmann, Prof. Dr. Alfred Zippelius, Prof. Dr. Walter Weber, Dr. Marcus Vetter, Dr. Sacha Rothschild, Dr. Julia Landin
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Isolating circulating tumor cells from the blood of cancer patients

The analysis of circulating tumor cells (CTCs) is an exceptional opportunity to study the biology of human cancer metastasis from minimally invasive biopsies, i. e. blood samples. In collaboration with Prof. Christoph Rochlitz, Prof. Viola Heinzelmann, Prof. Alfred Zippelius, Prof. Walter Weber, Dr. Marcus Vetter, Dr. Sacha Rothschild and Dr. Julia Landin at the University Hospital Basel, we routinely isolate and characterize CTCs from a variety of patients with metastatic cancers (e. g. breast, ovarian and lung cancer). Upon isolating CTCs from blood specimens with microfluidics technology, we process them for single cell resolution sequencing of their genome and transcriptome, to gain insights into the metastatic process. Further, we have implemented a protocol for deriving primary cultures from human CTCs, and use these as a model to study individualized drug susceptibility (i. e. so-called personalized medicine), as well as to study the requirement of specific genes for the metastatic process in xenograft models. With our approach, we aim to establish state-of-the-art and clinically relevant tools that will enable the identification of key vulnerabilities of cancer cells during the metastatic process.

Selected Publications

Gkountela S, Szczerba B, Donato C, Aceto N. (2016) Recent advances in the biology of human circulating tumor cells and metastasis. ESMO Open – Cancer Horizons. In press

Sarioglu AF*, Aceto N*, Kojic N, Donaldson, MC, Zeinali M, Hamza B, Engstrom A, Zhu H, Sundaresan TK, Miyamoto DT, *et al.* (2015) A microfluidic device for label-free, physical capture of circulating tumor cell clusters. Nat Methods 12, 685–691. *Equal contribution

Aceto N, Bardia A, Miyamoto DT, Donaldson MC, Wittner BS, Spencer JA, Yu M, Pely A, Engstrom A, Zhu H, *et al.* (2014) Circulating tumor cell clusters are oligoclonal precursors of breast cancer metastasis. Cell 158, 1110–1122

Yu M, Bardia A, Aceto N, Bersani F, Madden MW, Donaldson MC, Desai R, Zhu H, Comaills V, Zheng Z, *et al.* (2014) Cancer therapy. *Ex vivo* culture of circulating breast tumor cells for individualized testing of drug susceptibility. Science 345, 216–220

Tao JJ, Castel P, Radosevic-Robin N, Elkabets M, Auricchio N, Aceto N, Weitsman G, Barber P, Vojnovic B, Ellis H, *et al.* (2014) Antagonism of EGFR and HER3 enhances the response to inhibitors of the PI3K-Akt pathway in triple-negative breast cancer. Sci Signal 7, ra29

Tumor heterogeneity, metastasis and resistance

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Mechanisms of breast tumor heterogeneity, metastasis and resistance

Breast cancer is diagnosed in ~1.5 million women worldwide and ~500,000 lives are lost to the disease annually, the vast majority due to metastasis. Curing metastatic breast cancer represents an unmet medical need. Patients may do well after surgery and adjuvant treatment but drug-resistant, fatal metastases often develop. Critical to the phenomenon of resistance is tumor heterogeneity and this is the thread connecting the research in our lab.
At the molecular, cellular, and whole organism levels, we assess mechanisms that influence normal and neoplastic breast stem cells, metastasis, and resistance to therapy. We explore both cell autonomous (genetics, epigenetic, and proteomic) and non-cell autonomous mechanisms (immune cells, adipcoytes, etc).
We use systems medicine quantitative methods, unbiased pooled shRNA, CRISPR, transposon-based screens, and hypothesis-driven approaches. Computational biology is a very important part of our research. Moreover, we use multiphoton intravital imaging to assess the interactions between cancer cells and immune cells. These interdisciplinary projects seek to elucidate the integrated effects of signaling pathways and epigenetics on breast cell fate and tumor heterogeneity, and to leverage this mechanistic understanding into therapy. With clinicians from the University Hospital of Basel, we are building a breast cancer personalized medicine program wish should ultimately improve treatment for patients (Fig. 1).
M. Bentires-Alj is the founder and president of the European Network for Breast Development and Cancer (www.enbdc.org) that fosters global interactions between labs in these areas, and co-founder with Profs. Walter Paul Weber, Gerhard Christofori and Christoph Rochlitz of the Basel Breast Consortium (www.BaselBC.org), which is committed to promoting local basic, clinical, and translational interdisciplinary research projects within Switzerland.

Molecular mechanisms controlling normal and neoplastic breast stem cells:
PIK3CA^{H1047R} induces multipotency and multi-lineage mammary tumors. Two major cell lineages organized in a bi-layered structure constitute the mammary gland epithelium: the luminal layer lining the ducts and the alveoli and the myoepithelial layer with a basal location. A key issue in breast cancer biology is the effect of genomic lesions in specific mammary cell lineages on tumor heterogeneity and progression. The impact of transforming events on fate conversion in cancer cells-of-origin and thus their contribution to tumor heterogeneity remains largely elu-

Fig. 1: Research topics in the Bentires-Alj lab (https://bentireslab.org/)

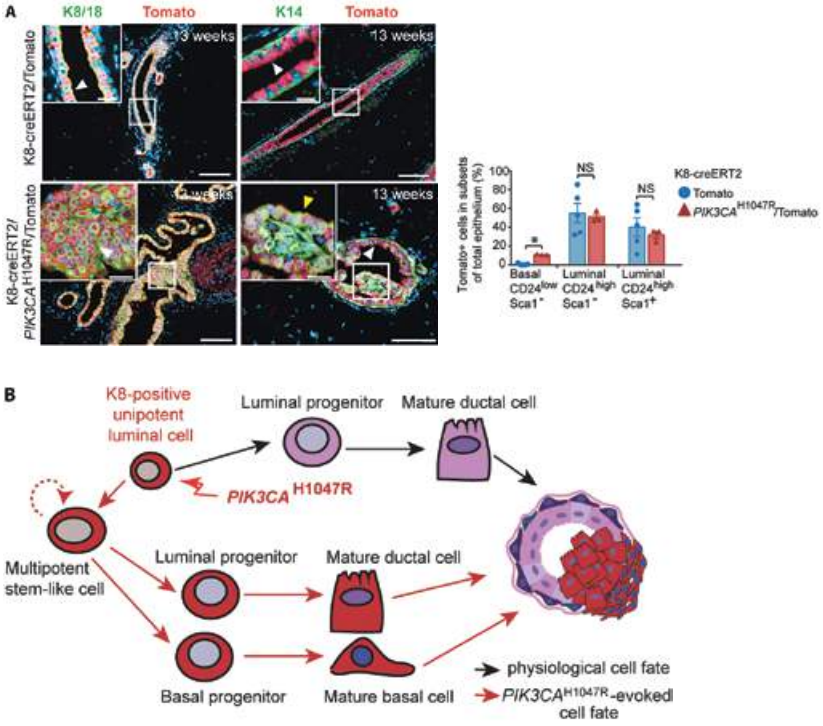

Fig. 2: Mutant PIK3CA induces mammary cell plasticity.
A. Representative images of 13-week tracing and FACS quantification of Tomato-positive mammary epithelial basal ($CD24^{Low}Sca1^{-}$) and luminal ($CD24^{low}Sca1^{-/+}$) subsets from K8-CreERT2/Tomato ($n=5$) and K8-CreERT2/*PIK3CA*^{H1047R}/Tomato mice (n=3) indicating that the expression of *PIK3CA*^{H1047R} induces cell plasticity. White arrowheads indicate luminal and yellow arrowheads indicate basal Tomato-labelled cells. Scale bars, 100 µm, 20 µm (magnifications). *$P<0.05$; NS: not significant
B. Model of the effect of *PIK3CA*^{H1047R} on cell fate in preneoplastic mammary glands. Under physiological conditions K8-positive luminal cells contribute to the homeostasis of luminal cells in the adult mammary gland (black arrows). Expression of *PIK3CA*^{H1047R} in cells results in dedifferentiation into a multipotent stem-like state from which cells further differentiate to basal and luminal mammary epithelial cells, contributing to mostly mixed-lineage malignant tumors (red arrows).

sive. Using in situ genetic lineage tracing and limiting dilution transplantation, we have unraveled the potential of PIK3CA^{H1047R}, one of the most frequent mutations occurring in human breast cancer, to induce multipotency during tumorigenesis in the mammary gland. Our results define a key effect of PIK3CA^{H1047R} on mammary cell fate in the pre-neoplastic mammary gland and show that the cell-of-origin of PIK3CA^{H1047R} tumors dictates their malignancy, thus revealing a mechanism underlying tumor heterogeneity and aggressiveness (Fig. 2) (Koren *et al.*, Nature 2015).

Molecular mechanisms controlling metastasis:
Discontinuation of CCL2 inhibition accelerates breast cancer metastasis by promoting angiogenesis. We have discovered a paradoxical effect of the CC chemokine ligand 2 (CCL2) in metastatic breast cancer. Secretion of CCL2 by mammary tumors recruits CCR2-expressing inflammatory monocytes to primary tumors and metastatic sites, and CCL2 neutralization in mice inhibits metastasis by retaining monocytes in the bone marrow. Surprisingly, interruption of CCL2 inhibition leads to an overshoot of metastases and accelerates death. This is the result of monocyte release from the bone marrow, enhancement of cancer cell mobilization from the primary tumor, as well as blood vessel formation and increased proliferation of metastatic cells in the lungs in an IL-6/VEGF-A-dependent manner. Our results call for caution when considering anti-CCL2 agents as monotherapy in metastatic disease and highlight the tumor microenvironment as a critical determinant of successful anti-metastatic therapy (Bonapace *et al.*, Nature 2014).

Connection to Clinical Practice

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Selected Publications

Koren S, Reavie L, Couto JP, De Silva D, Stadler MB, Roloff T, Britschgi A, Eichlisberger T, Kohler H, Aina O, *et al.* (2015) PIK3CA(H1047R) induces multipotency and multi-lineage mammary tumours. Nature 525, 114–118

Sausgruber N, Coissieux MM, Britschgi A, Wyckoff J, Aceto N, Leroy C, Stadler MB, Voshol H, Bonenfant D, Bentires-Alj M. (2015) Tyrosine phosphatase SHP2 increases cell motility in triple-negative breast cancer through the activation of SRC-family kinases. Oncogene 34, 2272–2278

Koren S, Bentires-Alj M. (2015). Breast Tumor Heterogeneity: Source of Fitness, Hurdle for Therapy. Molecular cell 60, 537–546

Ramos P, Bentires-Alj M. (2015) Mechanism-based cancer therapy: resistance to therapy, therapy for resistance. Oncogene 34, 3617–3626

Bonapace L, Coissieux MM, Wyckoff J, Mertz KD, Varga Z, Junt T, Bentires-Alj M. (2014) Cessation of CCL2 inhibition accelerates breast cancer metastasis by promoting angiogenesis. Nature 515, 130–133

Tumor Biology

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Molecular dissection of malignant tumor progression, metastasis and therapy resistance

The vast majority of cancer patient deaths are due to the systemic dissemination of cancer cells throughout the body and the seeding and outgrowth of secondary tumors (metastases) in distant organs. One major objective of our research is the identification and characterization of those cancer cells that are able to initiate and complete the metastatic process and to overcome current cancer therapies. In particular, we focus on the molecular mechanisms underlying the transition from benign tumors to malignant cancers and the metastatic dissemination of tumor cells. Moreover, we have set out to delineate the genes and pathways that allow cancer cells to evade from therapy. In addition to cultured tumor cell lines *in vitro*, we employ transplantation and transgenic mouse models of specific cancer types to determine causal connections between the expression of particular genes and tumor progression, metastasis and drug resistance *in vivo.*

The development of malignant tumors is in part characterized by a tumor cell's capability to overcome cell-cell adhesion and to invade surrounding tissue by a process referred to as epithelial-mesenchymal-transition (EMT). An EMT underlies the conversion of epithelial, differentiated cells to mesenchymal, migratory and invasive cells. In the past years, we have learned that an EMT occurs in multiple stages and is regulated by sophisticated molecular networks regulating the expression of a large number of protein, lncRNA and miRNA-encoding genes. More recently, we have noted that an EMT also selects for cancer cells exhibiting hallmarks of cancer stem cells and increased drug resistance. Notably, we have identified a large number of transcription factors that act as master regulators not only in the initiation and execution of the morphogenic process of an EMT but also in providing survival signals to cancer cells and thus allowing cancer cells to seed and grow metastases in distant organs. We investigate the direct target genes of these transcription factors and their role in tumor metastasis. We also assess the role of miRNAs and lnRNAs and their target genes in the regulation of an EMT and of metastatic dissemination. With these experimental approaches we aim at identifying the master regulators of an EMT and cancer metastasis and we plan to scrutinize their potential as therapeutic targets for preventing metastatic disease.

In a second line of research, we investigate the molecular pathways underlying the development of evasive resistance to targeted cancer therapy. We employ a number of cultured cancer cell lines and mouse models to study the pathological, physiological and molecular consequences of therapies targeting tumor angiogenesis and malignant tumor progression. In particular, we use cell biological, biochemical and bioinformatical analysis to delineate the molecular pathways allowing cancer cells to escape from targeted therapy. Recently, we have found that tumors shift their metabolism to glycolysis and acquire a status of metabolic symbiosis between individual cells of a tumor to overcome anti-angiogenic therapy. Finally, in collaboration with pharmaceutical companies we are investigating the efficacy and biological consequences of various anti-angiogenic and anti-metastatic cancer treatments.

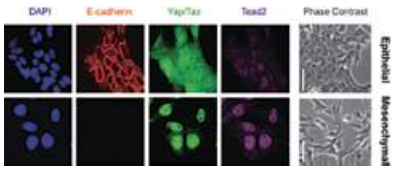

Fig. 1: Tead2 upregulation and Yap/Taz subcellular localization during EMT. Morphological differences between epithelial and mesenchymal counterparts of murine breast cancer cells (phase contrast, scale bar, 50 µm). Immunofluorescent staining of Tead2 and its co-factors Yap and Taz shows their increased expression and nuclear translocation in mesenchymal cells where they activate the expression of genes involved in EMT and metastasis. E-cadherin staining shows epithelial cell junctions. DAPI was used to visualize nuclei (scale bars, 25 µm; Diepenbruck, Waldmeier *et al.*, 2014).

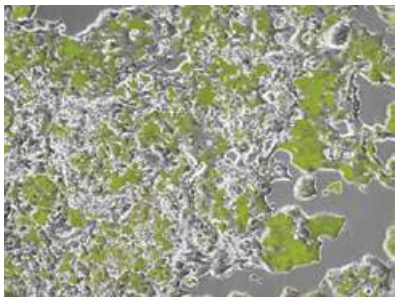

Fig. 2: IGF-II production as a public goods games network. The microscopic picture shows a co-culture of cancer cells which do not produce IGF-II (colorless cells) and cancer cells which express IGF-II (green cells). Both cell types require IGF-II for their survival and they compete against each other to reach a specific homeostasis of producer cells and consumer cells. Guess who will win (answer: Archetti *et al.*, 2015).

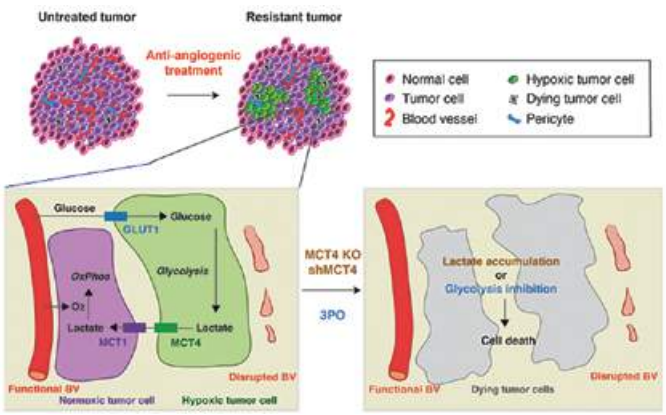

Fig. 3: Targeting metabolic symbiosis overcomes resistance to anti-angiogenic therapy. Metabolic symbiosis as a mechanism underlying evasive resistance to anti-angiogenic therapy by the multi-kinase inhibitors nintedanib and sunitinib. Inhibition of glycolysis by 3PO or genetic ablation of the lactate exporter MCT4 in tumor cells disrupts metabolic symbiosis, overrides therapy resistance, and suppresses tumor growth (Pisarsky, Bill *et al.*, 2016).

Selected Publications

Pisarsky L, Bill R, Fagiani E, Dimeloe S, Goosen RW, Hagmann J, Hess C, Christofori G. (2016) Targeting metabolic symbiosis to overcome resistance to antiangiogenic therapy. Cell Reports 15, 1161–1174

Bill R, Fagiani E, Zumsteg A, Antoniadis H, Johansson D, Albrecht I, Hilberg F, Christofori G. (2015) Nintedanib is a highly effective therapeutic for neuroendocrine carcinoma of the pancreas (PNET) in the Rip1Tag2 transgenic mouse model. Clinical Cancer Res. 21, 4856–4867

Archetti M, Ferraro D, Christofori G. (2015) Heterogeneity for IGF-II production maintained by public goods dynamics in neuroendocrine pancreatic cancer. Proc. Natl. Acad. Sci. USA 112, 1833–1838

Diepenbruck M, Waldmeier L, Ivanek R, Berninger P, Arnold P, van Nimwegen E, Christofori G. (2014) Tead2 expression levels control Yap/Taz nuclear localization and epithelial-mesenchymal transition. J. Cell Sci. 127, 1523–1536

Fantozzi A, Gruber DC, Pisarsky L, Heck C, Kunita A, Yilmaz M, Meyer-Schaller N, Cornille K, Hopfer U, Bentires-Alj M, Christofori, G. (2014) VEGF-mediated angiogenesis links EMT-induced cancer stemness to tumor initiation. Cancer Res. 74, 1566–1575

Connection to Clinical Practice

Markus Heim, Walter Weber
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Evasive resistance to targeted therapy and tumor heterogeneity

The development of resistance to targeted cancer therapy (evasive resistance) has appeared a major obstacle in treating the disease. In two network projects connecting patient care (Markus Heim), biomedical research (Mike Hall, Biozentrum) and computational biology (Niko Beerenwinkel and Jörg Stelling, D-BSSE, ETHZ, Basel), we aim at the molecular dissection of the pathways underlying the development of drug resistance to current cancer therapy. We have generated drug-sensitive cell lines and their drug-resistant counterparts and novel transgenic and patient-derived xenografted (PDX) mouse models of hepatocellular carcinoma (HCC) which recapitulate the development of HCC in patients. These cellular and animal models are now being used for molecular, biochemical and genomic analysis of the processes underlying evasive resistance and to test first alternative therapies to overcome evasive resistance. These projects are supported by a European Research Council (ERC) Synergy Grant and by a SystemsX.ch MTD Grant.

In a second network project, in collaboration with the Department of Surgery of the University Hospital Basel (Walter Weber) and basic and computational researchers of the Friedrich-Miescher-Institute in Basel (Mohammed Bentires-Alj and Michael Stadler), the University of Zürich (Bernd Bodenmiller) and IBM Rüschlikon (Maria Rodriguez), we address basic questions regarding breast cancer cell heterogeneity in patients and in experimental models and aim at identifying the cancer-propagating, metastatic breast cancer cell population and learn about the genetic programs driving these cells. This project is funded by a SystemsX.ch MTD Grant.

Human Genomics

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Dr. Per Hoffmann (Research Associate)
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Molecular genetic analysis of neuropsychiatric disorders, hereditary colorectal cancer syndromes, and congenital developmental disorders

Our research group aims to identify the molecular (genetic) basis of human diseases by combining human genetics knowledge, new genomics technologies, bioinformatics/-statistical approaches, and in-depth phenotyping. We work on complex neuropsychiatric disorders (Sven Cichon and Per Hoffmann), congenital developmental disorders (Isabel Filges, primarily linked to the DKF), and hereditary colorectal cancer syndromes (Karl Heinimann).

Neuropsychiatric disorders

We recently published the so far largest genome-wide association study (GWAS) of bipolar disorder (BD) (Mühleisen *et al.*, 2014), a common neuropsychiatric disorder and implicated novel risk loci at the ADCY2 gene and between the genes MIR2113 and POU3F2. In particular the gene for ADCY2 (encoding adenylate cyclase 2) is biologically interesting, it plays a key role in cAMP-dependent G-protein coupled receptor pathways. Disturbed neurotransmission at these pathways is a long-standing hypothesis in psychiatric research.
We contributed to the largest GWAS of schizophrenia to date (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014) in which 128 independent single nucleotide polymorphisms (SNPs) were identified. Using these GWAS data, we performed several follow-up analyses, including an analysis of the contribution of microRNA coding genes to BD (Forstner *et al.,* 2015).
A current project aims at the identification of risk genes for BD in large, multiply affected BD and major depression families, by analyzing whole-exome sequencing data in up to 10 genetically distant patients selected from each family.

Congenital developmental disorders

Our goal is to understand the genomic basis of congenital developmental disorders and improve patient care. We identified several genes causing developmental delay and intellectual disabilities (ID) through the systematic study of individuals with unexplained congenital anomaly syndromes and syndromic and non-syndromic ID (e.g. PTCHD1, SETBP1, SMARCA2). Recent research expands to using next generation sequencing technologies to discover genes in which mutations cause early fetal mal-development, since improved ultrasound technology and its use by maternal fetal medicine specialists fetal diagnosis clinics worldwide deal with an increasing number of cases with serious or lethal anomalies of unknown cause. Most important findings so far were the delineation of the first human lethal phenotype caused by mutations in KIF14 (Filges *et al.,* 2014), and the identification of mutations in CENPF, causing a variable phenotypic presentation ranging from a fetal lethal ciliary phenotype to the postnatal Stømme syndrome (Filges *et al.,* 2016).

Fig. 1a

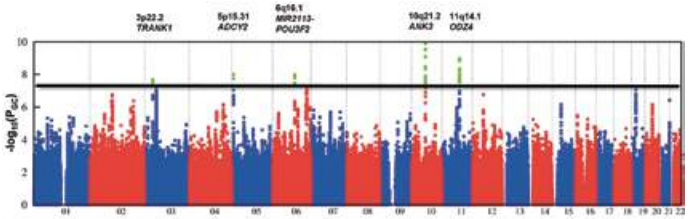

Fig. 1b

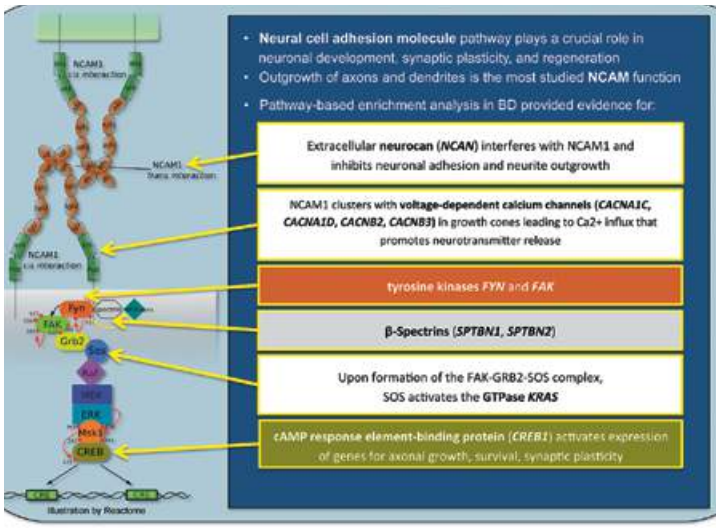

Fig. 1: Refers to our research on neuropsychiatric disorders. In **Fig. 1a,** results of our most recent (and so far largest) GWAS for bipolar disorder (BD) are shown (Mühleisen *et al.*, 2014). The Manhattan plot gives a genome-wide overview of association results for SNPs. The x-axis depicts all the whole genome from chromosome 1 to X. The y-axis shows the negative decadic logarithm of the p-value for each tested SNP. 56 SNPs exceeded the threshold for genome-wide significance ($p<5 \times e-08$), they clustered in 5 genomic loci: chr. 3 containing the TRANK1 gene, chr. 5 covering the ADCY2 gene, chr. 6 in an intergenic region between genes MIR2113 and POU3F2, chr. 10 covering the ANK3 gene, and chr. 11 including the gene ODZ4. **Fig. 1b** shows results of a biological pathway analysis using the program INRICH, using the complete GWAS results as input. Our analysis shows that association signals in SNPs located in genes coding for proteins of the NCAM1 signaling pathway are significantly clustered. This provides evidence that the NCAM1 signaling pathway is disturbed in BD. Future studies will have to show the exact functional consequences (pathophysiology) of such SNP risk alleles on the pathway in BD (Manuscript in preparation).

Selected Publications

Filges I, Bruder E, Brandal K, Meier S, Undlien DE, Waage TR, Hoesli I, Schubach M, de Beer T, Sheng Y, Hoeller S, Schulzke S, Røsby O, Miny P, Tercanli S, Oppedal T, Meyer P, Selmer KK, Strømme P. (2016) Strømme Syndrome Is a Ciliary Disorder Caused by Mutations in CENPF. Hum. Mutat. 37, 359–63

Blatter RH, Plasilova M, Wenzel F, Gokaslan ST, Terracciano L, Ashfaq R, Heinimann K. (2015) Somatic Alterations in Juvenile Polyps from BMPR1A and SMAD4 Mutation Carriers. Genes Chrom. Cancer 54, 575–582

Kovac MB, Kovacova M, Bachraty H, Bachrata K, Piscuoglio S, Hutter P, Ilencikova D, Bartosova Z, Tomlinson I, Roethlisberger B, Heinimann K. (2015) High-Resolution Breakpoint Analysis Provides Evidence for the Sequence-Directed Nature of Genome Rearrangements in Hereditary Disorders. Hum. Mutat. 36, 250–259

Forstner AJ, Hofmann A, Maaser A, Sumer S, Khudayberdiev S, Mühleisen TW, Cichon S, Nöthen MM. (2015) Genome-wide analysis implicates microRNAs and their target genes in the development of bipolar disorder. Transl. Psychiatry 5, e678

Mühleisen TW, Leber M, Schulze TG, Strohmaier J, Degenhardt F, Treutlein J, Cichon S. (2014) Genome-wide association study reveals two new risk loci for bipolar disorder. Nat. Commun. 5, 3339.

Hereditary colorectal cancer syndromes

We have assessed the mutational processes behind large, genomic deletions/insertions leading to colorectal cancer syndromes. Little is known about genomic rearrangements (GRs) in the germ line of cancer patients. We investigated DNA motifs and higher order structures of genome architecture, which may result in losses and gains of genetic material in the germ line, and created an algorithm to predict the propensity of rearrangements (Kovac *et al.,* 2015).

Another focus was on juvenile polyposis syndrome (JPS) with SMAD4 or BMPR1A germline mutations (1st-hit). Little is known about the nature of somatic alterations (2nd-hit) in SMAD4-/BMPR1A related juvenile polyps. We screened polyps from three patients with SMAD4-/BMPR1A germ line mutations for somatic alterations and SMAD4 protein expression. No somatic alterations were identified in 14 SMAD4-related polyps. SMAD4 protein expression, however, was lost in 57 % of the polyps (6 showing concomitant loss in both epithelial and stromal compartments). In BMPR1A-related polyps, five out of nine (56 %) displayed gene copy number neutral LOH, which had occurred in the epithelial compartment. The heterogeneity of genetic mutations and protein expression levels indicates that different modes of gene inactivation can be operational in SMAD4- and BMPR1A-related polyp formation. The observation that half of BMPR1A-related polyps displayed LOH suggests that BMPR1A acts as a tumour suppressor gene (Blatter *et al.,* 2015).

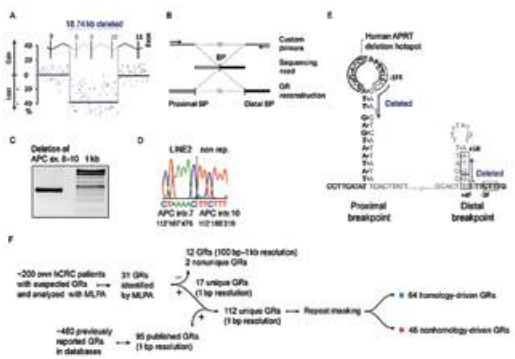

Fig. 2: Showing some of the work peformed in Hereditary colorectal cancer syndromes. In particular, this figure shows how Karl Heinimann investigated the mutational processes behind large, genomic deletions/insertions leading to colorectal cancer syndromes (Kovac *et al.* 2015). The figure gives an overview of the fine-mapping and the inclusion scheme for patients with genome rearrangements. Sections A–E exemplify fine-mapping of a 18.7 kb deletion encompassing APC exons 8–10 by custom-tiled array CGH, (A–C) allele-specific PCR followed by a GR reconstruction, (D) breakpoint sequencing, and (E) structural analysis and motif identification. Section F depicts selection and sub-classing of 112 nonrecurrent genomic rearrangements based on the repeat masking annotations. BP: breakpoint, GR: genome rearrangement.

Ovarian Cancer Research

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Identification of novel molecular signatures to improve epithelial ovarian cancer outcome

Gynecological cancers in general and ovarian cancer (OC) in particular are the main focus of our research. OC is the fifth most common cause of death from all cancers in women and the leading cause of death from gynecological malignancies, with a poor prognosis (5-year survival <20%). Major issues with OC are its *heterogeneity*, its *unclear genetic origin,* its *diagnosis at advanced stages* due to the lack of accurate biomarker, its *ability to rapidly metastasize* into other organs, and its *insufficient treatment* due to disease recurrence *(therapy resistance)* and *missing personalized therapy regimens.* Our **particular focus** is to: (i) identify molecular/genetic signatures that unequivocally discriminate OC patients by their clinico-pathological parameters (e.g. histotype, grade, stages) and identify molecular targets for the better prediction of disease outcome and for the design of targeted therapies. (ii) elucidate the molecular basis and biological functions of aberrantly glycosylated proteins and lipids in cancer initiation, progression, and dissemination. (iii) evaluate in clinical studies novel means to improve diagnosis and treatment of gynecological cancers.

Identification of discriminating signatures in the era of omics (Prof. Viola Heinzelmann)

Identification of novel "discriminating" diagnostic and prognostic markers and therapeutic targets is an urgent need in the fight against cancer. Employing various glycan-based immunoassays we identified sets of specific markers (glycans) that enable us to discriminate healthy controls from ovarian cancer patients with sensitivity and specificity comparable to the current biomarker CA125 (Jacob et al, 2012, 2014; Pochechueva et al, 2011, 2014, 2016). We also found particular glycan-based signatures (glycans and glyco-genes) which distinguish serous ovarian from peritoneal cancers: this, together with transcriptomic and epidemiological data, provides further evidence that these two cancers are clearly different diseases and hence should no longer be clinically treated as one (manuscript in preparation). We also identified two protein kinases with significance in ovarian cancer. ROR2, a Wnt-signaling-associated receptor tyrosine kinase, is overexpressed in ovarian cancer patients (tissue microarray) and is implicated in proliferation, migration, and invasion (Henry et al, 2015). MELK (maternal embryonic leucine-zipper kinase) was identified as an interesting candidate in ovarian cancer (Heinzelmann-Schwarz et al, 2004) already in 2004 when still known as KIAA0175. We now show in broad transcriptomic/bioinformatical data analyses that MELK expression is elevated in ovarian cancer patients, increases with ascending aggressiveness, and correlates with poor disease outcome, and that its inhibitor OTSSP167 is highly active against (including drug-resistant) ovarian cancer cells (manuscript in revision).

Regulation and function of specific glycan motifs in ovarian cancer (Glyco-Oncology: Dr. Francis Jacob)

It is acknowledged that glycosylation is crucial to the proper function(s) of glycoproteins and glycosphingolipids but the molecular and biological details are poorly understood. Owing the expertise of Francis Jacob as Glycobiologist the "Glyco-Oncology" branch within Ovarian Cancer Research was launched in 2015. In collaboration with Nicki Packer (Sydney), membrane protein glycan features ("bisecting GlcNAc" type *N*-glycans) unique to ovarian cancer cells were identified and experimental evidence is provided that expression of *MGAT3* (enzyme for bisecting GlcNAc synthesis) is epigenetically regulated by DNA-methylation and correlates with presence of bisecting GlcNAc on glycoproteins (Anugraham *et al.,* 2014, Kohler *et al.,* 2016). Likewise, evaluation of 18 TCGA cancer types (6118 sam-

ples) demonstrated a generally poor overall survival in cancer patients with high MGAT3 expression but interestingly identified among ovarian cancer a subgroup of "long-term survivors" with low MGAT3-expression **(Figure).** We also identified by glycan-based immunoassays, mass spectrometry, and flow cytometry a subset of glycosphingolipids with possible roles in ovarian cancer, including cell migration (Jacob *et al.,* 2014, Alam *et al.,* 2015, Anugraham *et al.,* 2015). Further *in vitro* and *in vivo* investigations address the role(s) of glycosphingolipids in epithelial-mesenchymal transition and drug responses using specifically designed (CRISPR/Cas9-edited) "glyco-gene knockout" ovarian cancer cells (manuscripts in preparation).

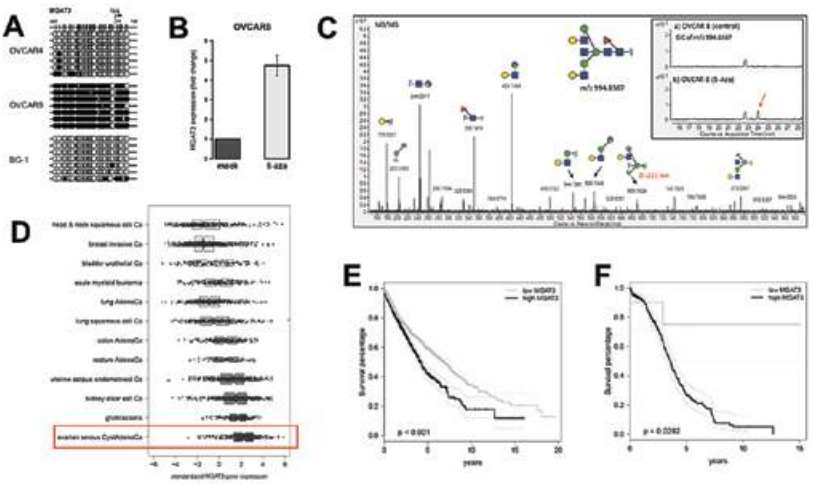

Figure: MGAT3 transcription start site is hypermethylated (black circles) in OVCAR8 and poorly methylated (white) in OVCAR4 and BG1 cells **(A)** Demethylation by 5-Aza in hypermethylated OVCAR8 recovers MGAT3 expression **(B)** and bisecting GlcNAc expression **(C:** red arrow in insert). Boxplot of *MGAT3* expression in TCGA PANCAN12 data set (*n*=3587): lowest expression in head and neck squamous cell cancer (top) to highest in ovarian serious cystadenocarcinoma (red frame) **(D).** Kaplan-Meier: low MGAT3 expression (grey line) associates with poor survival (various cancers combined) **(E).** Some high-grade serous ovarian cancer patients have low MGAT3 expression (grey) and are "long-term survivors" **(F).** Kohler *et al.,* 2016.

Selected Publications

Kohler RS, Anugraham M, López MN, Xiao C, Schoetzau A, Hettich T, Schlotterbeck G, Fedier A, Jacob F, Heinzelmann-Schwarz, V. (2016) Epigenetic activation of MGAT3 and corresponding bisecting GlcNAc shortens the survival of cancer patients. Oncotarget, 26, 51674–51686

Manegold-Brauer G, Buechel J, Knipprath-Mészaros A, Schoetzau A, Hacker NF, Tercanli S, Lapaire O, Heinzelmann-Schwarz V. (2016) Improved Detection Rate of Ovarian Cancer Using a 2-Step Triage Model of the Risk of Malignancy Index and Expert Sonography in an Outpatient Screening Setting. Int. J. Gynecol. Cancer 26, 1062–1069

Henry C, Llamosas E, Knipprath-Mészaros A, Schoetzau A, Obermann E, Fuenfschilling M, Caduff R, Fink D, Hacker N, Ward R, Heinzelmann-Schwarz V, Ford C. (2015) Targeting the ROR1 and ROR2 receptors in epithelial ovarian cancer inhibits cell migration and invasion. Oncotarget 6, 40310–40326

Jacob F, Anugraham M, Pochechueva T, Tse BW, Alam S, Guertler R, Bovin NV, Fedier A, Hacker NF, Huflejt ME, Packer N, Heinzelmann-Schwarz VA. (2014b) The glycosphingolipid P_1 is an ovarian cancer-associated carbohydrate antigen involved in migration. Br. J. Cancer 111, 1634–1645

Anugraham M, Jacob F, Nixdorf S, Everest-Dass AV, Heinzelmann-Schwarz V, Packer NH. (2014) Specific glycosylation of membrane proteins in epithelial ovarian cancer cell lines: glycan structures reflect gene expression and DNA methylation status. Mol. Cell. Proteomics 13, 2213–2232

Connection to Clinical Practice

Prof. Viola Heinzelmann-Schwarz
Hospital for Women, Department of Gynecology and Gynecological Oncology, University Hospital Basel

Towards improved detection, diagnosis, prediction, and management of gynecological malignancies

The risk of malignancy index (RMI), which allows appropriate preoperative triaging of patients with malignant ovarian tumors and accurate planning of the required surgical procedure, in some cases remains inconclusive. With our "improved" RMI, which includes a mathematical two-step model incorporating expert pattern recognition, we were able to particularly identify previously undetected ovarian cancer patients (Manegold-Brauer *et al.,* 2014, 2016). One study showed that women with a positive family history generally use mammography screening more often and perceive changes in the breast earlier than women without such history (Schwab et al, 2014) and another confirms the causal relationship between persistent infection with high-risk HPV genotypes and vulvar and cervical cancer (Heinzelmann-Schwarz *et al.,* 2014). Preliminary results from Mito/Mango 16b and INOVATYON clinical trials initiated in 2014 are pending.

Cancer Immunotherapy

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Tumor-host interactions in human colorectal cancer

Colorectal cancer (CRC) is a leading cause of cancer-related death. Non-transformed cells in CRC microenvironment, including tumor-associated mesenchymal stromal cells (TASCs) and immune cells, have been recognized to play key roles in disease progression. Whereas infiltration by specific immune cell types is significantly associated with prolonged patient survival, TASC abundance predicts unfavorable prognosis. Mechanisms leading to recruitment of these cell populations and underlying their effects on clinical outcome remain to be clarified.
CRC arises in an environment populated by the gut microflora. Commensal bacteria translocate across the dysfunctional neoplastic epithelium into the lamina propria, thus possibly stimulating stromal and immune cells. The potential impact of these events on tumor development and progression remains to be fully elucidated.
We are interested in investigating interactions occurring between tumor, stromal and immune system in CRC and their modulation by the gut microflora. Understanding the complex network of tumor-host interactions in CRC may allow the identification of novel prognostic biomarkers and potential new areas of therapeutic intervention.

Main Projects

Role of CRC infiltrating IL-17-producing T cells: Phenotypes and prognostic relevance of tumor infiltrating IL-17-producing T cells (Th17) in CRC are still debated. Upon *ex vivo* analysis, and *in vitro* and *in vivo* experiments we found that CRC infiltrating Th17 are polyfunctional effector cells able to produce, in addition to IL-17, a spectrum of cytokine/chemokines ultimately leading to recruitment of beneficial CD8+ T cells and neutrophils. Our study reveals a positive role played by tumor infiltrating Th17 in CRC, thus calling for caution when envisaging novel IL-17/Th17 targeted therapies.

Monocytes-Th17 cells crosstalk: Monocytes (Mo) promote differentiation of naïve cells into Th17. However, their impact on pre-differentiated Th17 cells, such those infiltrating CRCs, is unknown. We assessed the ability of classical (cMo) and non-classical monocytes (ncMo) to promote expansion of memory Th17 cells *in vitro*. We found that in the absence of microbial stimulation ncMo are more efficient stimulators of Th17 than cMo, and their ability is counteracted by LFA-1/ICAM-1 interaction. These data highlight ncMo as potential new therapeutic targets in IL-17-mediated inflammation.

Immune cell recruitment into CRC: Chemotactic factors leading to CRC infiltration by beneficial immune cells are still unclear. Upon *ex vivo* analysis of human CRC specimens, we identified a panel of chemokine genes underlying tumor infiltration by favorable immune cell subsets. Stimulation of CRC cells by gut microbiota markedly enhanced the expression of these chemokines *in vitro* and *in vivo*, and led to increased T cell recruitment into tumor xenografts. Importantly, in human CRC specimens, bacterial loads correlated with chemokine expression levels and extent of T cell infiltration. Our findings identify the gut microbiota as critical modulator of immune cell trafficking into CRCs.

Impact of TASCs on CRC progression: Mechanisms underlying the negative prognostic significance of TASCs in CRC are not fully understood. By *in vitro* and *in vivo* experiments, we found that upon tumor conditioning, TASCs acquire surface TGF-β expression and induce epithelial-to-mesenchymal transition (EMT) in CRC cells (see Figure 1). This results in higher numbers of circulating tumor cells, ultimately leading to increase metastasis formation. These data reveal a novel mechanism of tumor-stroma interaction and may suggest novel therapeutic interventions.

3D culture models for primary CRC tissues: In collaboration with the Tissues Engineering group, we developed an innovative 3D system, based on a perfused bioreactor, for culturing freshly isolated CRC specimens. This system proved capable of preserving all components of CRC microenvironment, including tumor, mesenchymal and immune cells, up to five days, and might therefore be suitable for testing the efficacy of innovative anti-cancer compounds targeting the tumor or the tumor-associated stroma.

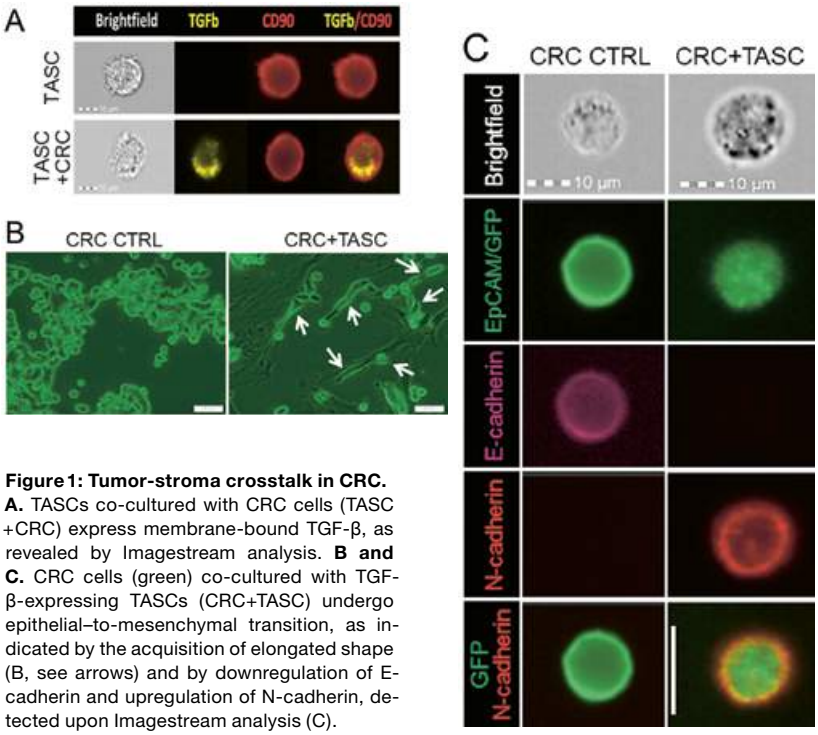

Figure 1: Tumor-stroma crosstalk in CRC. **A.** TASCs co-cultured with CRC cells (TASC +CRC) express membrane-bound TGF-β, as revealed by Imagestream analysis. **B and C.** CRC cells (green) co-cultured with TGF-β-expressing TASCs (CRC+TASC) undergo epithelial–to-mesenchymal transition, as indicated by the acquisition of elongated shape (B, see arrows) and by downregulation of E-cadherin and upregulation of N-cadherin, detected upon Imagestream analysis (C).

Selected Publications

Amicarella F, Muraro MG, Hirt C, Cremonesi E, Padovan E, Mele V, Governa V, Han J, Huber X, Droeser RA, *et al.* (2015) Dual role of tumor infiltrating T-helper 17 cells in human colorectal cancer. Gut. 2017, 66:692–704. (doi: 10.1136/gutjnl-2015-310016)

Traunecker E, Gardner R, Fonseca J, Polido-Pereira J, Seitz M, Villiger PM, Iezzi G, Padovan E. (2015) Blocking of LFA-1 enhances expansion of Th17 cells induced by human CD14(+) CD16(++) nonclassical monocytes. Eur. J. Immunol. 45:1414–25

Hirt C, Papadimitropoulos A, Muraro MG, Mele V, Panopoulos E, Cremonesi E, Ivanek R, Schultz-Thater E, Droeser RA, Mengus C, *et al.* Bioreactor-engineered cancer tissue-like structures mimic phenotypes, gene expression profiles and drug resistance patterns observed "*in vivo*" (2015). Biomaterials 62:138–46

Mele V, Muraro MG, Calabrese D, Pfaff D, Amatruda N, Amicarella F, Kvinlaug B, Bocelli-Tyndall C, Martin I, Resink TJ, *et al.* (2014) Mesenchymal stromal cells induce epithelial-to-mesenchymal transition in human colorectal cancer cells through the expression of surface-bound TGF-beta. Int. J. Cancer 134:2583–94

Sadallah S, Amicarella F, Eken C, Iezzi G, Schifferli J. (2014) Ectosomes released by platelets induce the differentiation of CD4+ T cells into T regulatory cells. Thromb Haemost 112:1219–29

Connection to Clinical Practice

Prof. Daniel Oertli
Department of Surgery, University Hospital Basel

Immunotherapeutic intervention in human colorectal cancer

The Cancer Immunotherapy group is closely connected to the Department of Surgery of the University Hospital Basel, led by Prof. Daniel Oertli. Several surgeons, including young doctors in training, have been involved in the planning and development of our research projects. Our ultimate goal is the identification of novel targets for immunotherapeutic intervention in colorectal cancer.

Furthermore, we have established a collaborative network with the surgical units of other Swiss hospitals, including St. Claraspital Basel (Dr. M. Bolli), Kantonsspital Olten (Prof. Markus Zuber), Kantonsspital Arau (Prof. Walter Marti), Kantonsspital St. Gallen (Dr. Michel Adamina), and Ospedale Civicio di Lugano (Prof. Raffaele Rosso), ensuring regular access to clinical samples.

We have also established a proficient collaboration with the Institute of Pathology, of the University of Basel. The availability in this unit of the tissue-microarray technology has allowed the rapid evaluation of the clinical relevance of putative novel prognostic markers on large cohorts of patients. Furthermore, the mutual exchange of specific know-hows has resulted in the generation of significant synergies.

Dermatology

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Genodermatoses as a clue to cancer development

Genodermatoses are inherited skin diseases, some of them with a high impact on life quality. We are doing research on genodermatoses with an increased risk to or a remarkable frequency of non-melanoma skin cancer (NMSC), the most common skin cancer in the human general population. Most frequent NMSC are basal cell carcinomas (BCC) and cutaneous squamous cell carcinoma (cSCC). We focus our studies on epidermodysplasia verruciformis (EV) and ichthyosis with confetti (IWC), and aim to improve the knowledge on correlation of mutation and development of NMSC.

EV

Epidermodysplasia verruciformis (EV) is a rare autosomal recessively inherited genodermatosis with about 500 patients described in literature. EV patients develop plane wart-like lesions mainly on neck and extremities during childhood and have a high risk for early development of NMSC (Fig. 1). Patients have an increased susceptibility to specific human papilloma virus (HPV), usually beta-HPV. These HPV are harmless for the general population because they miss a specific gene named E5. E5 is supposed to be necessary for HPV to overcom the human immune system. About 60 % of EV patients present bi-allelic mutations in the genes *TMC6*/*EVER1* or *TMC8*/*EVER2.* Function of both proteins and pathomechanism in EV are unknown. Since EV patients are prone to infections by E5-missing HPV it is assumed that TMC6 and TMC8 are part of innate immune system. Immunocompromised patients after organ transplatation have a 60-fold increased risk for development of NMSC compared to the immunocompetent population. We hypothesized that rare SNPs in one of both *TMC* genes are correlated to their risk, but our investigations of renal transplant cohort from Basel could not confirm such a correlation. Our team was able to expand the phenotypical spectrum of EV by careful examination of patients. Investigations of both *TMC* genes revealed new mutations and we characterized the stability of correlated mRNA. Identification and analysis of an undescribed gene in EV by the group from J-L Casanova (Rockefeller University, NY; INSERM and Imagine Institute Paris, France) in collaboration with our group will help to characterize pathomechanisms beyond EV.

IWC

IWC is an ultra-rare autosomal dominant inherited genodermatosis with less than 50 patients described in literature. IWC patients are born with an erythematous scaling skin (Fig. 2A). During childhood patients develop thousands of white spots on their skin which look like normal skin (Fig. 2B). By conscientious clinical examination of largest patient group we defined additional clinical features such as malformation of ears (Fig. 2C) and hypoplastic mammillae (Fig. 2D). Those specific

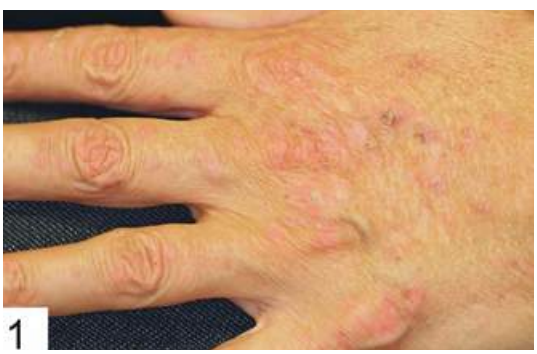

Fig. 1: Plane wart-like lesions typical in EV are present on the left hand of a patient.

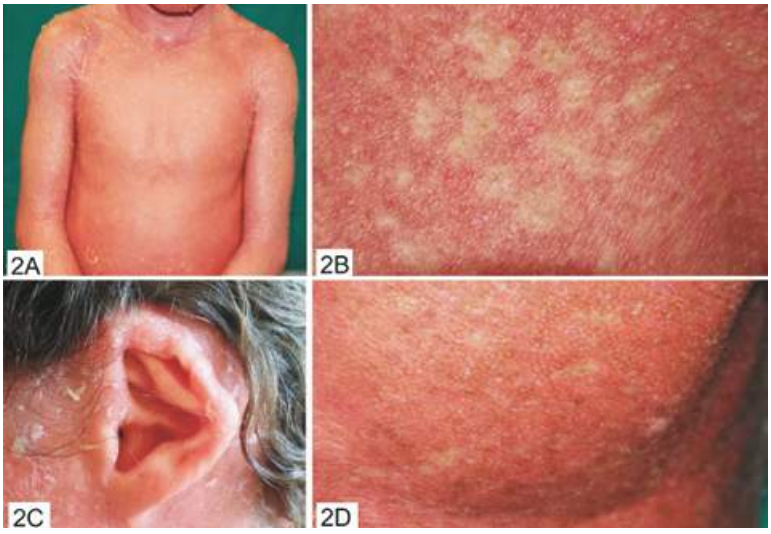

Fig. 2A: IWC patients are born with an erythematous and scaly skin covering the whole body. **B.** Later on life, usually during early childhood, white spots arise which are typical for IWC. **C.** Clinical features which may help to differentiate IWC from other ichthyoses are malformation of ears and **D.** hypoplastic mamillae.

characteristics may help to distinguish IWC patients from other erythematous ichthyoses before development of typical white spots. Patients with IWC carry a heterozygous mutation in the 3'-end of *keratin 10 (KRT10)* which leads to an arginine-rich C-terminus in the resulting protein instead of glycin-richness. Presumably that switch in charge induces a nuclear signal of the aberrant keratin 10 (K10) resulting in a nuclear accumulation instead of cytoplasmic localisation. In epidermal keratinocytes of IWC patients, but not in the underlying dermis as we could show, lots of mitotic recombinations or gene conversions occur on the chromosome with the *KRT10* gene. That leads to a loss of heterozygosity (LOH) of the mutation without loss of copy number and results in keratinocytes which express only wildtype KRT10. Those cells present as white spots on patients' skin. Even though examined IWC patients carried various mutations and the detected number of arginine differed relevant, no genotype-phenotype correlation could be defined. In contrast to EV, patients with IWC are not excessively reported to develop cSCC, but single reports of early NMSC development exists. Since the disease is ultra-rare estimation of NMSC risk is very difficult. Future research of our group aims for identification of the mechanism underlying the disease and leading to a prognosis for the patients regarding their tumour risk, especially on the skin.

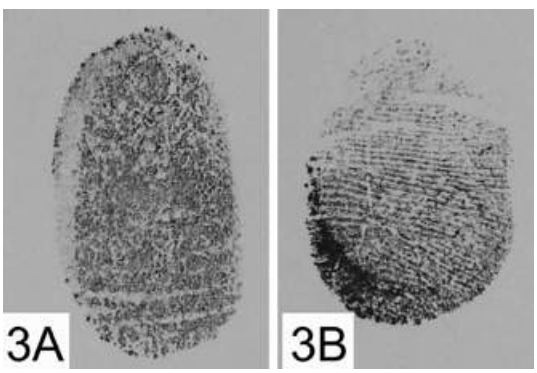

Fig. 3A: Adermatoglyphia is a rare phenotype which can be sign of a syndrome or occur as an isolated feature. In that case it is caused by specific mutations in *SMARCAD1.* **B.** Healthy control.

Connection to Clinical Practice

Molecular investigation of genetically determined skin diseases

Our research focuses on rare genetic skin diseases which could function as a model for general mechanisms. Most impact is applied to skin carcinoma development with the aim to understand basic mechanisms and identify new targets for tumour therapy. Patients who suffer from the related disease are under medical treatment in the Clinic of Dermatology. Since all of our research activities is close-by the needs of the patients we also examine single families outside of carcinoma topics. For instance, we could analyse the germline mutation in a family without fingerprints. In cooperation with an Israeli dermatological research group we identified a specific splice variant of *SMARCAD1* as a transcript responsible for development of fingerprints on human palms and soles (Fig. 3).

The knowledge of the underlying cause of their skin disease is important for the patients, not only for estimation of cancer risk but also for the interpersonal relationships as skin is an important mediator between human individuals.

Selected Publications

Spoerri I, Brena M, De Mesmaeker J, Schlipf N, Fischer J, Tadini G, Itin PH, Burger B. (2015) The phenotypic and genotypic spectra of ichthyosis with confetti plus novel genetic variation in the 3' end of KRT10. From disease to a syndrome. JAMA Dermatol, 151: 64–69

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Burger B, Itin PH. (2014) Epidermodysplasia Verruciformis. Curr Probl Dermatol. 45: 123–131

Nousbeck J, Sarig O, Magal L, Warshauer E, Burger B, Itin P, Sprecher E. (2014) Mutations in SMARCAD1 cause autosomal dominant adermatoglyphia and perturb the expression of epidermal differentiation-associated genes. Br J Dermatol. 171(6): 1521–1524

Eytan O, Qiaoli L, Nousbeck J, van Steensel MAM, Burger B, Hohl D, Taieb A, Prey S, Bachmann D, Avitan-Hersh E, Chung HJ, Shemer A, Trau H, Bergman R, Fuchs-Telem D, Warshauer E, Israeli S, Itin PH, Sarig O, Uitto J, Sprecher E. (2014) Increased epidermal expression and absence of mutations in CARD14 in a series of sporadic PRP patients. Br J Dermatol. 170(5):1196–8

Bruegger C, Spoerri I, Arnold AW, Itin PH, Burger B. (2013) MicroRNA expression differs in cutaneous squamous cell carcinomas and healthy skin of immunocompetent individuals. Exp Dermatol. 22: 426–428

Brain Tumor Biology

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Glioma development: from biomarker identification to molecular mechanisms

Gliomas are among the deadliest cancers, with median survivals varying between months for the malignant grade IV glioblastoma (GBM), to decades for low-grade glioma (LGG). Gliomas progress by brain tissue invasion. The aim of our Laboratory is to understand mechanisms underlying glioma invasion. This involves the identification of biomarkers, genetic regulators, signaling networks and molecular effectors of invasion that can ultimately be targeted to control glioma progression. Through an active exchange between clinics and our laboratory, we are collecting freshly resected glioma biopsies for genotyping and *ex vivo* cell culture. In parallel, we are entering personal, clinical, imaging, histopathological and molecular annotations to construct a comprehensive glioma patient database. This information is useful for classifying gliomas into molecular subsets and allowing identification of biomarkers that may reveal novel glioma pathways.

IDH mutations in low-grade gliomas

IDH neomorphic mutation (*IDH*mut), found in 80% LGG, catalyzes α-ketoglutarate conversion into 2-hydroxyglutarate, accumulation of which maintains CpG methylation. This results in *MGMT* epigenetic silencing and *TP53* CpG-to-CpA mutational transition. We stratified 210 LGG according to these molecular criteria in a retrospective study. Although *IDH*mut status is associated with a lower risk of death (*HR*death=0.35, *P*=0.0023), *IDH*mut subsets consistently showed higher risks of malignant transformation (MT) than of death. This supports the finding that molecular events relevant to *IDH* mutation impacts early glioma development prior to MT (see Leu *et al.,* 2016).

The interplay of 3q26 clustered genes *SOX2, PIK3CA, MFN1* and *OPA1* in GBM cell invasion

Chromosome band 3q26, frequently altered in GBM, contains the genes for transcription factor SOX2, growth factor/AKT signaling activator PIK3CA, and MFN1 and OPA1, two effectors of mitochondria fusion, a process linked to inhibition of cell motility (Fig. 1A). We aimed at determining the roles of these four genes in GBM cell invasion.
Compared to parental LN319 GBM cells, individual 3q26 gene knock-downs consistently shortened mitochondria, and enhanced cell invasion (Fig. 1BC). These phenotype similarities suggested that these 3q26 genes act on a common invasion pathway. Pharmacological inactivation of AKT, downstream of PIK3CA, impairs SOX2 nuclear localization and aggravates SOX2 turnover (Fig. 2A). Chromatin im-

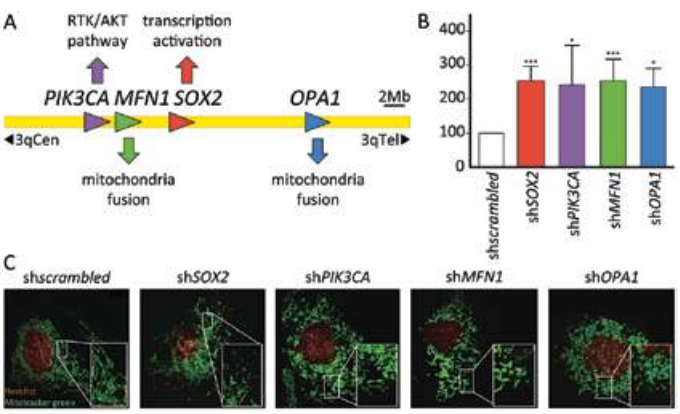
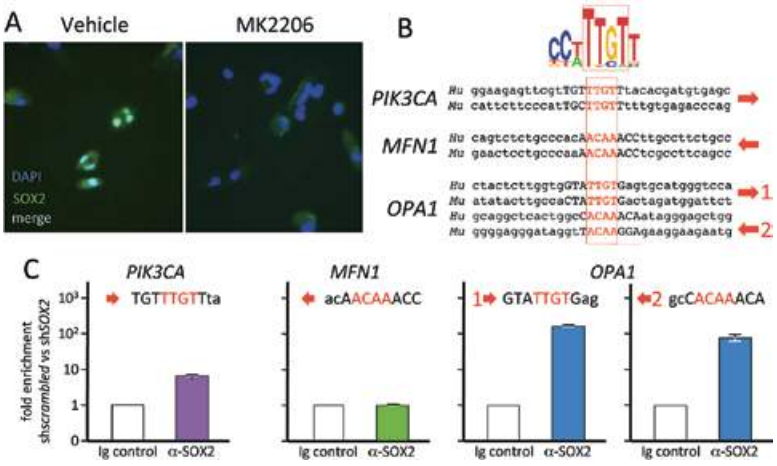


Fig. 1

Fig. 2



muno-precipitation and luciferase reporter gene assays show that SOX2 *trans*-activates *PIK3CA, MFN1* and *OPA1* (Fig. 2BC). This indicates a positive regulation loop where AKT signaling activates SOX2 function, which in turn activates *PIK3CA, MFN1* and *OPA1* transcription. Copy number variations at 3q26 analyzed in 100 glioma biopsies show frequent *SOX2* gain (29%) and *OPA1* loss (32%) (Fig. 3A). *SOX2* amplification is consistent with enhanced transcriptional activation of oncogenic targets such as *PIK3CA.* In contrast, *OPA1* needs to be lost to counteract the impact of SOX2 on mitochondria fusion and invasion suppression. Thus, among the genes trans-activated by SOX2, are oncogenic and tumor suppressor genes. While the oncogenic ones *(PIK3CA)* are kept *'on',* OPA1 may need to be turned *'off'* by deletion. Thus, we provide evidence that a regional interplay between 3q26 genes promotes glioma invasion (Fig. 3B). Copy number variations of 3q26 genes suggest optimization of these oncogenic activities and are currently being tested for their impact on tumor invasion in glioma patients (see connection to clinical practice).

Combined expression of nestin and SPARC identifies isolated astrocytoma cells in brain tissue

Identification of individual invasive glioma cells in brain tissue requires markers that specifically recognize tumor cells. We tested the presence of proteins involved in glioma development (proliferation, survival, invasion, differentiation, transcription and metabolism) on a tissue micro-array that contains brain sections at various distances from the tumor core and various glioma histology and grade. Glial progenitor marker nestin together with secreted acidic and rich in cysteine (SPARC) represent a specific combination for recognizing glioma cells in a non-neoplastic environment (see Aljammal *et al.,* 2015).

Connection to Clinical Practice

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Prof. Christoph Stippich
Departments of Neurosurgery and Neuro-Radiology

Interdisciplinarity between neurosurgery and neuro-imaging

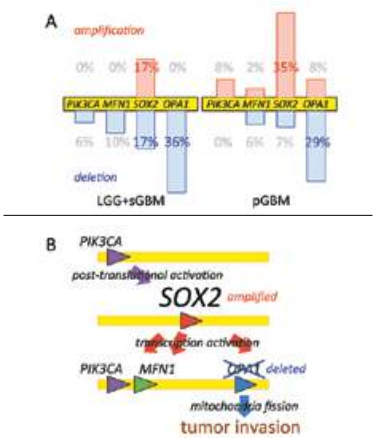
The collaboration between the Departments of Neurosurgery and Neuroradiology aims at studying the impact of the volume of tumor components on the overall survival of glioblastoma patients.

This project consists of the retrospective neuro-imaging analysis of 64 glioblastoma patients operated in our department for which the following imaging parameters are assessed:

(i) volumes of enhancing, non-enhancing, necrosis and edema domains from the pre-operative magnetic resonance images; (ii) preoperative Response Assessment in Neuro-Oncology (RANO); and (iii) postoperative enhancing residual volume. Multivariable cox proportional hazard models will be used to associate the different tumor components with overall survival adjusting for the potential confounders: age and postoperative enhancing residual tumor. Preliminary results suggest a statistically significant multiplicative effect of RANO on the hazard for death.

As a clinical extension on our project on the 3q26 gene cluster, we are also testing whether 3q26 genetic alterations may correspond to specific tumor patterns such as the volume and the type of recurrence (local vs. distant), and the focality (unifocal vs. multifocal).

Fig. 3



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Cell migration and neurito-genesis

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Spatio-temporal regulation of cell signaling during cell migration and neuronal differentiation

The ability of vertebrate cells to directionally migrate is critical to development, the immune response and wound healing, and its regulation is compromised in pathologies such as metastatic cancer and vascular disease. The capacity of neurons to directionally extend neuronal processes is crucial for the proper wiring of the brain. Both processes take advantage of a tight spatio-temporal control of cytoskeletal and adhesion dynamics, with signaling events that operate on length and time scales of single microns and tens of seconds. One current limitation is that these biologically relevant scales are not accessible with traditional biochemical and cell biological approaches. We are broadly interested in different signaling networks regulating the two processes mentioned above with the focus to design and implement novel technologies to grasp their spatio-temporal dynamics at relevant biological scales.

Genetically-encoded biosensors to measure signaling events in time and space

We have devised a novel toolkit to rapidly construct genetically-encoded, fluorescence resonance energy transfer-based biosensors for a wide variety of signaling molecules. Our approach enables to visualize micrometric signaling domains that fluctuate of time scales of tens of seconds. By example, the GTPase RhoA is specifically activated at the tip of F-actin bundles in neuronal growth cone filopodia (Fig. 1) or at the leading edge of migrating fibroblasts. Rac1 and Cdc42 are activated at overlapping but distinct regions within the growth cone. This degree of precision cannot be matched by any biochemical measurement. In the case of the MAP kinase ERK, the biosensor revealed signaling noise within a population of cells, which was not previously accessible using western blot-based measurements cell population averages. Previous work has proposed that duration of the pERK signal in response to different growth factors regulate cell fate decision such as differentiation or proliferation. We observe that these growth factor-induced signaling responses are extremely heterogeneous when analyzed at the single cell level (Fig. 2). This explains the phenotypic "fate" noise observed in a population of cells: a given growth factor will not lead to homogeneous proliferation or differentiation within the cell population, but rather a mix of multiple behaviours. Thus, our biosensors provide a novel approach to understand signaling dynamics at relevant biological scales.

Local mRNA translation during neurite outgrowth

We have performed a genome-wide screen for mRNAs enriched within neuronal growth cones. We have found that the MKK7 mRNA, which encodes a MAPKK for JNK, is locally translated within the growth cone. This leads to specific activation of JNK within the neurite, where it regulates microtubule bundling necessary for robust neurite outgrowth (Fig. 3). This provides a spatio-temporal signaling mechanism to specifically couple JNK signaling to regulation of microtubules, and to uncouple it from regulation of cellular stress.

Spatio-temporal signaling programs during neuronal guidance

We are currently studying a large signaling network of 220 neurite-localized proteins that regulate the cytoskeleton, identified using a proteomic approach. siRNA-mediated knockdown of these proteins only leads to very subtle phenotypes that can only be grasped using timelapse imaging of the neurite outgrowth process. For that purpose, we have combined high content live cell imaging, computer-vision based image and statistical analyses, and identified a number of regula-

tory networks regulating neurite initiation, extension, branching, collapse, etc. This emphasizes the need of a system biology approach to understand these complex networks.

Fibroblast cell migration. We have identified a highly persistent fibroblast migration mode. We observe that specific cytoskeletal structures act as a spatial organizer, that allows to constantly polarize the cell and to specify different subcellular zones involved in membrane protrusion or tail retraction. We have identified a leading edge-localized, collision sensor, that allows to sense when two migrating cells encounter each other.

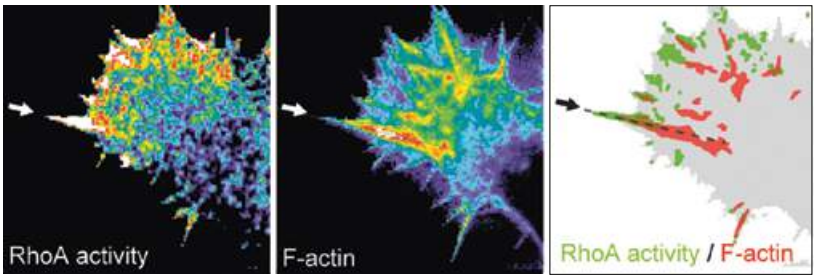

Fig. 1

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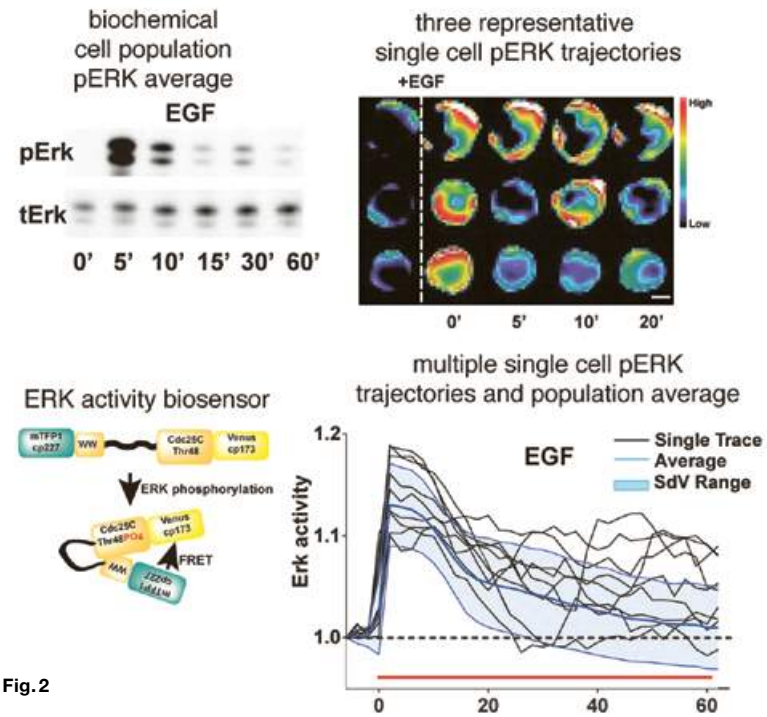

Fig. 2

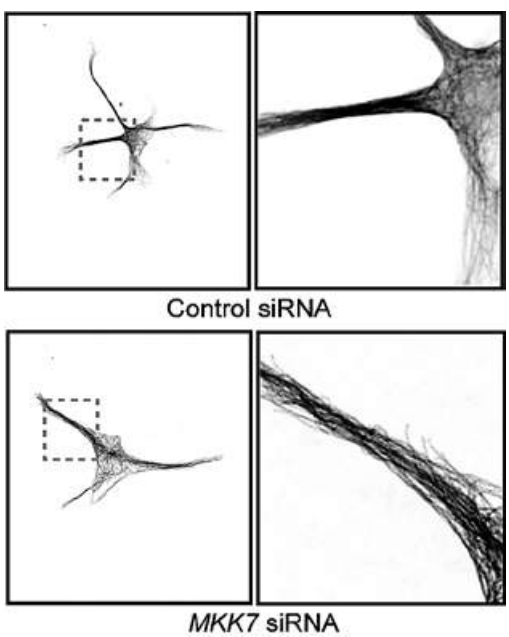

Fig. 3

Genome Plasticity

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Genome and epigenome dynamics in development, aging and disease

Reactive agents of endogenous and environmental origin pose a continuous threat to the integrity of genomes. By chemical modification of the DNA, they alter its coding properties and promote genetic mutation. Such “damage” to DNA, however, does not only occur randomly, through chemical reactions, but also by the action of enzymes, in which case the purpose is to increase genetic variance or alter cell fate determining epigenetic signatures, i.e. DNA methylation. Modifications of either kind occur thousands of times in our DNA every day and need to be controlled if genome function is to be maintained. We investigate the molecular mechanisms underlying this dynamic instability of genomes. A main research focus of the past years has been the role of DNA repair in active DNA demethylation and its contribution to the patterning and maintenance of epigenetic programs – hence – cell identity. We have been following three main lines of investigation directed towards unraveling the basic molecular mechanisms and function of active DNA demethylation, the relevance of DNA methylation control and stability for human aging and disease, and the impact of the environment on the stability of DNA methylation.

(Epi)genetic maintenance by DNA repair

A long-standing focus of our research has been the biological function of an enigmatic DNA repair pathway operating through the “Thymine DNA Glycosylase” (TDG). TDG first caught our attention because of its ability to hydrolyze thymine or uracil from T•G and U•G DNA mismatches. These mismatches arise frequently in genomic DNA by deamination of cytosine or 5-methylC (5mC) and, unless repaired, will generate C>T mutations, the most prevalent DNA change found in cancers. Thus, its enzymatic activity clearly implicates TDG in the anti-mutagenic repair of these mismatches, but this function has never been corroborated by biological evidence. The entry point for our recent research was the discovery that a defect in TDG causes developmental failure in a mouse model, due to aberrant DNA methylation patterning. Together with work of others on trans-eleven-translocation (TET) proteins, these findings indicated that TET and TDG constitute a long-sought pathway for active DNA demethylation, operating through oxidation of 5mC by TET and replacement of the oxidized 5mC with a C through TDG dependent DNA repair. We then established that TDG and TET cooperate in differentiating cells to drive cyclic methylation and demethylation events at specific gene regulatory sequences. On the mechanistic side, we were able to show that TET1 and TDG physically and functionally interact to form an active DNA demethylase and to provide proof by biochemical reconstitution that the TET-TDG-repair system, coordinated by SUMO modification, is capable of productive and coordinated DNA demethylation. Ongoing work addresses, amongst other questions, the involvement of non-coding RNAs in assembling DNA demethylation complexes in chromatin.

DNA Methylation Dynamics in Aging and Disease

Aberrant DNA methylation contributes to tumorigenesis by deregulating the genome. Exactly why, how and when methylation changes arise during carcinogenesis is unknown. Our aim is to identify genetic and environmental conditions controlling DNA methylation stability in human tissues and assess the underlying mechanisms. We started by investigating the stability of DNA methylation in the aging healthy human colon. Using a molecular epidemiological approach, we were able to identify distinct patterns of age-dependent and cancer-relevant DNA methylation drift and found that the rate of such changes is modulated by exposure to lifestyle factors such as medication and BMI. This work allowed us for the first time

to derive true cancer-specific DNA hypermethylation signatures and to precisely characterize subtypes of colorectal cancer with and without CpG-island methylator phenotpype (CIMP). We were then able to show that CIMP in these cancers is associated with a failure in active DNA demethylation through the TET1-TDG pathway, caused by BRAF-induced downregulation of TET1, hence linking oncogenic signaling with epigenetic remodeling.

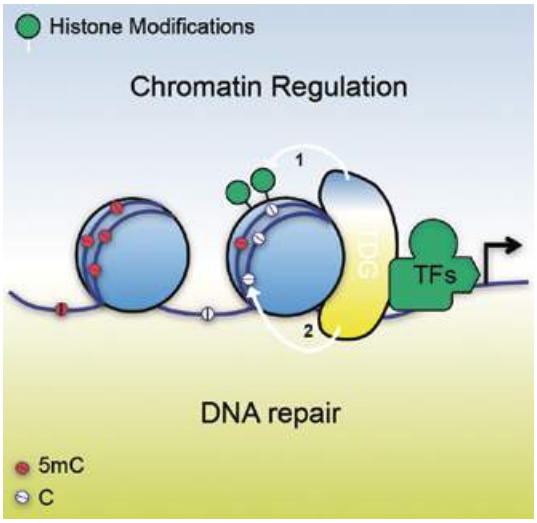

Fig. 1: TDG dependent DNA excision repair controls epigenetic states through DNA demethylation.

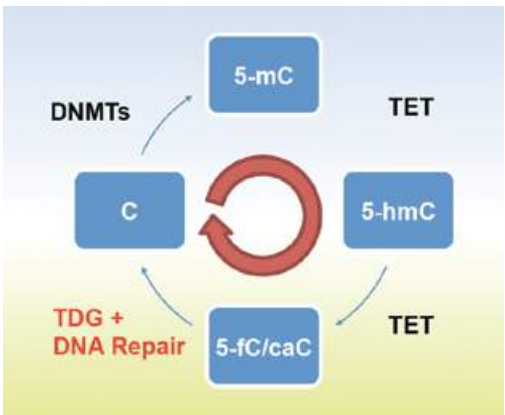

Fig. 2: TDG and TET hydroxylases cooperate in cyclic DNA methylation and active oxidative demethylation at CpG di-nucleotides in the genome. TDG excises 5-fC and 5-caC, thereby initiating excision repair incorporating an unmethylated C. 5-mC, 5-methylcytosine; 5-hmC, 5-hydroxymC; 5-fC, 5-formylC; 5-caC, 5 carbocylC.

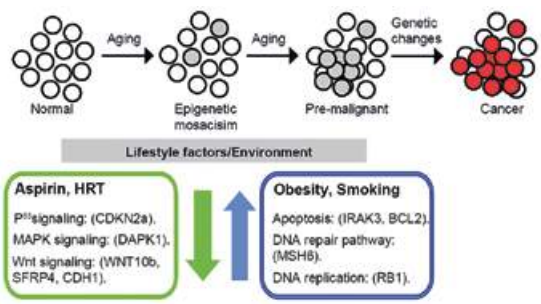

Fig. 3: Lifestyle factors modulate the rate of DNA methylation drift in the aging colonic mucosa and, by inference, early events of colorectal carcinogenesis.

Connection to Clinical Practice

PD Dr. Kaspar Truninger
Gastroenterologie Oberaargau, DBM

Truninger is a Gastroenterologist working with us 20% (since 12 years) on his own expenses.

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Noreen F, Röösli M, Gaj P, Pietrzak J, Weis S, Urfer P, Regula J, Schär P, Truninger K. (2014) Modulation of age- and cancer-associated DNA methylation change in the healthy colon by aspirin and lifestyle. JNCI J Natl Cancer Inst (2014) 106(7): dju161

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Liu Y, Duong W, Krawczyk C, Bretschneider N, Borbély G, Varshney M, Zinser C, Schär P, Rüegg J. (2016) Oestrogen receptor β regulates epigenetic patterns at specific genomic loci through interaction with thymine DNA glycosylase. Epigenetics Chromatin 9, 7

Childhood Leukemia

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Dissection of critical cellular and molecular mechanisms of acute myeloid leukemia (AML) to develop novel therapeutic strategies

The overall goal of our research is to functionally dissect critical molecular mechanisms that drive the development and maintenance of acute myeloid leukemia (AML). Our work currently focuses on genetic alterations of epigenetic regulators such as mixed lineage leukemia (MLL) or nucleoporin-98 (NUP98) often associated with poor disease outcome. NUP98 is recurrently involved in chromosomal translocations leading to expression of fusions with multiple partner genes. We have cloned full-length cDNAs of several NUP98-fusions including NUP98-NSD1 and NUP98-MLL. In contrast to others, we found that overexpression of NUP98-NSD1 is not sufficient to transform hematopoietic stem and progenitor cells, but rapidly induces AML in mice when expressed in combination with the FLT3-ITD mutation, which is present in tumor cells from the majority of NUP98-NSD1+ patients (Thanasopoulou *et al.*, 2014). In parallel, we found that expression of transforming NUP98 fusions resulted in unprecedented alterations of the nuclear membrane (collaboration with B. Fahrenkrog, Bruxelles) (Fahrenkrog *et al.*, 2016).

In contrast to NUP98, the oncogenic potential of MLL fusion genes is well established. MLL-fusions form transcriptionally active large multi-protein complexes that bind to chromatin through adapter proteins like MENIN and LEDGF/p75. We used several *in vitro* and *in vivo* AML models to demonstrate potent anti-leukemic activities of several small molecules that inhibit critical co-factors of the MLL fusion complex such as the BET-protein BRD4 or the CBP/EP300 transcriptional co-regulators (collaboration with S. Knapp, Oxford/ Frankfurt) (Picaud *et al.*, 2015).

Whether the cell of origin influences the biology of AML is an ongoing matter of debates. We established conditional transgenic mouse lines for some of the most prevalent MLL fusions ("iMLL-AF9", "iMLL-ENL") that allow to model AML from long-term repopulating hematopoietic stem cells (LT-HSC) but also more committed progenitor cells such as granulocyte-macrophage progenitors (GMP). Activation of iMLL-AF9 in LT-HSC resulted in unusually invasive clonogenic growth in methylcellulose not observed upon activation in GMP. *In vivo*, activation of iMLL-AF9 upon transplantation of LT-HSC, induced in 10–20% of the recipients an invasive and chemoresistant AML after a very short latency. Interestingly, "LT-HSC-early iMLL-AF9" AML cells expressed many genes related to adhesion, migration and epithelial-mesenchymal transition (EMT) known from progressing solid cancers. Strikingly, about 20% of a large cohort of AML patients were characterized by similar gene expression signatures: akin to the mouse model, leukemic cells from these patients expressed high levels of the transcription factors EVI1 and ERG and the EMT-regulator ZEB1 (Fig. 1). Cross-species comparison revealed >100 genes that characterized aggressiveness and poor outcome in mouse and human AML, and may represent novel biomarkers and/or origin-related therapeutic targets (Stavropoulou *et al.*, 2016).

The iMLL-ENL mouse line allowed for the first time to closely model human mixed lineage leukemia associated with this fusion. In contrast to iMLL-AF9, iMLL-ENL preferentially transformed HSC rather than more committed progenitor cells. Interestingly, transformation by iMLL-ENL was dependent of the fusion exceeding endogenous Mll mRNA levels. Importantly, *MLL-ENL* mRNA levels exceeding *MLL* in leukemic blasts were also found in patients carrying this alteration. Collectively, these experiments suggested that transformation by MLL-ENL (and most likely other MLL fusions) depends on a critical fusion dose, and is significantly influenced by cell of origin within the hematopoietic hierarchy *(submitted).*

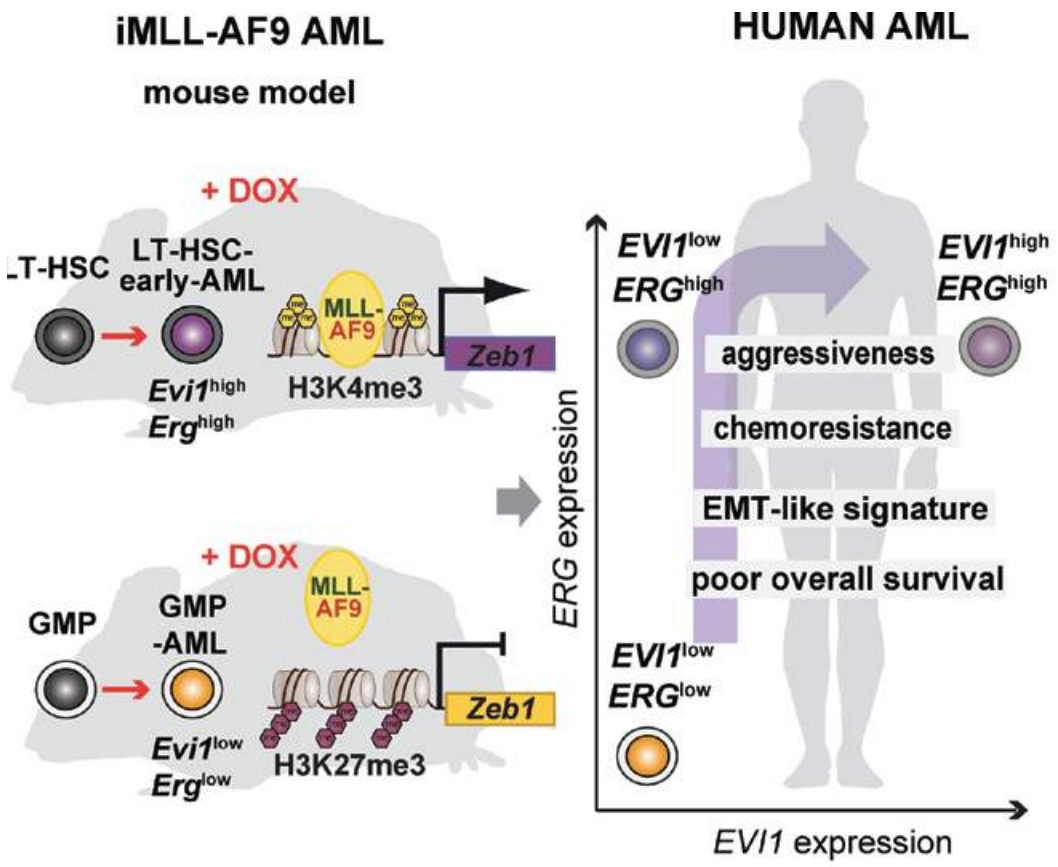

Fig. 1: Modeling of MLL-AF9-driven AML of different cellular origins in mice revealed striking similarities with the human disease. Induction of iMLL-AF9 in long-term hematopoietic stem cells (LT-HSC) resulted in a more aggressive disease than activation in granulocyte-macrophage progenitors (GMP). Invasive LT-HSC-derived AML was characterized by high expression levels of *Evi1* and *Erg,* and by an EMT-like gene expression signature with direct induction of *Zeb1.* Interestingly, high expression levels of *EVI1* and *ERG* also characterized AML patients with poor outcome suggesting a similar impact of the cellular origin on the biology of the disease in mouse and man.

Selected Publications

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Stavropoulou V, Kaspar S, Brault L, Sanders MA, Juge S, Morettini S, Tzankov A, Iacovino M, Lau IJ, Milne TA, *et al.* (2016). MLL-AF9 Expression in Hematopoietic Stem Cells Drives a Highly Invasive AML Expressing EMT-Related Genes Linked to Poor Outcome. Cancer Cell 30, 43–58

Oncology Surgery

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Innovative tools and concepts for the development of cancer immunotherapies and the analysis of human cancer immune contexture

Previous melanoma immunotherapy clinical trials by our group have emphasized the difficulties inherent in the generation and expansion of memory cells specific for human tumor associated antigens. Indeed, central memory CD8+ T-cells (TCM) have been shown to play key roles in protective immunity against infectious agents, cancer immunotherapy, and in adoptive treatments of malignant and viral diseases. However, CD8+ TCM generation is challenging and usually requires CD4+ CD40L+ T-cell "help" during the priming of naïve CD8+ T-cells. We have generated a replication incompetent CD40 ligand-expressing recombinant vaccinia virus (rVV40L) to promote the differentiation of human naïve CD8+ T cells into TCM specific for viral and tumor associated antigens. In the absence of CD4+ T cells, a single *in vitro* stimulation of naïve CD8+ T cells by rVV40L-infected non-professional CD14+ antigen presenting cells promotes the rapid generation of viral or tumor associated antigen specific CD8+ T-cells displaying TCM phenotype and functions. These observations strongly support the use of similar reagents for clinical immunization and adoptive immunotherapy purposes. Chronic inflammation plays decisive roles in cancer progression by powerfully shaping tumor microenvironment. In particular, tumor infiltration by myeloid cells has frequently been associated with poor prognosis in different types of cancer, including murine models of CRC. Granulocyte-monocyte colony-stimulating factor (GM-CSF, CSF2) is a powerful activator of myeloid cells. We have observed that GM-CSF induces in human macrophages the ability to inhibit the proliferation of CRC cells *in vitro*. Importantly, GM-CSF gene is expressed to significantly higher extents in CRC than in autologous healthy mucosa. By using a large (>1200) number of sporadic CRC specimens, accounting for >85% of human CRC, we have observed that in mismatch repair proficient (MMRp) cancers GM-CSF production by CRC cells is associated with improved survival in univariate and multivariate analyses. The favorable prognostic relevance of GM-CSF production by CRC cells is particularly evident in MMRp cancers where poor CD8+ T cell infiltration is detectable. These data underline specificities of human CRC immunobiology and indicate that prognostic significance of defined tumor micro-environmental features critically depends on tumor types and related anatomic districts. These data also urge the development of innovative *in vitro* models, utilizing human cells and sharing critical features of tumor progression *in vivo*. To address these issues we have used a perfused bioreactor to sustain CRC cell growth and to test established treatment regimens in an in-vitro setting as compared to normal cell culture or xenografts. By culturing HT29 cells in 3D on collagen scaffolds under direct perfusion tissue-like structures characterized by a heterogeneous pattern of proliferating cells and apoptotic cells could be successfully established closely resembling xenografts generated in immunodeficient mice. Upon perfusion, homogeneous seeding on scaffolds could be obtained and significantly higher numbers of tumour cells were recovered, as compared to static 3D cultures. Following treatment with clinically relevant concentrations of 5-FU no effect on numbers of cells cultured in 3D perfusion and as xenografts was observed, as compared to a marked inhibition of 2D cultures. Importantly, in perfused cultures we observed marginal effects on the expression of BCL-2, TRAF1, FLIP apoptosis resistance genes, as compared to a significant down regulation in 2D cell cultures and 3D static conditions. Accordingly, a combination of ABT-199, a new clinically approved BCL-2 inhibitor, and 5-FU induced additional cytostatic and cytotoxic effects in 3D perfused but not in 2D cell cultures. Thus, cultures in perfused bioreactors are characterized by sensitivity to

chemotherapeutic treatments similar to xenografts and significantly different from standard 2D cellular assays. 3D perfusion models could therefore help to improve the understanding of mechanisms related to drug resistance development and to evaluate new targets for treatment.

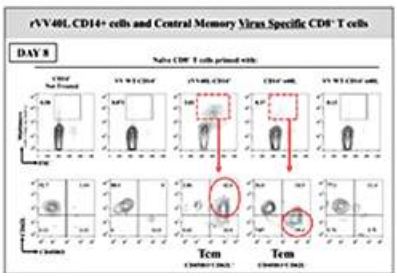

Fig. 1: Culture of naïve CD8+ cells in the presence of a recombinant vaccinia virus encoding CD40 ligand induces antigen specific central memory cells.
Peripheral blood CD8+ T cells from a vaccinia virus (VV) naive healthy donor were stimulated for 8 days in the presence of autologous CD14+ monocytes left untreated or infected with a wild type VV (VV WT) or a recombinant VV (rVV) expressing CD40 ligand (rVV40L). Additional controls included soluble CD40 ligand (sCD40L) and a combination of VV WT and sCD40L. Following culture, cells were stained with VV-specific multimers and CD45RO and CD62L specific markers, identifying central memory T cells (TcM).

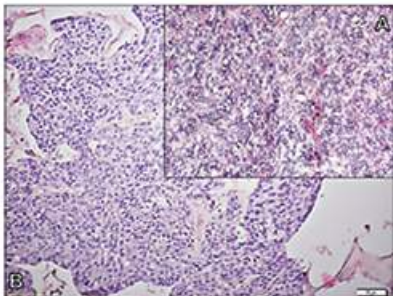

Fig. 2: Culture of tumor cells in a perfused bioreactor on collagen scaffolds results in the generation of tissue-like structures closely resembling *in vivo* growth in immunodeficient animals.
HT29 colorectal cancer (CRC) cells were injected subcutaneously in a NSG immunodeficient mouse (A) or cultured for 10 days on collagen scaffolds inserted in a perfused bioreactor (B). Tissue-like structures generated in the bioreactor are characterized by morphological features similar to those observed in *in vivo* growing cancer cells.

Selected Publications

Nebiker C, Han J, Eppenberger-Castori S, Iezzi G, Hirt C, Amicarella F, Cremonesi E, Huber X, Padovan E, Angrisani B, Droeser RA, Rosso R, Bolli M, Oertli D, von Holzen U, Adamina M, Muraro MG, Mengus C, Zajac P, Sconocchia G, Zuber M, Tornillo L, Terracciano L, Spagnoli GC. GM-CSF production by tumor cells is associated with improved survival in colorectal cancer. Clin Cancer Res 2014, 20:3094–3106

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Hirt C, Papadimitriopoulos A, Muraro MG, Mele V, Panopoulos E, Cremonesi E, Ivanek R, Schultz-Thater E, Droeser RA, Mengus C, Heberer M, Oertli D, Iezzi G, Zajac P, Eppenberger-Castori S, Tornillo L, Terracciano L, Martin I, Spagnoli GC. Bioreactor-engineered cancer tissue-like structures mimic phenotypes, gene expression profiles and drug resistance patterns observed *"in vivo"*. Biomaterials, 2015, 62:138–146

Trella E, Raafat N, Mengus C, Traunecker S, Governa V, Heidtmann S, Heberer M, Oertli D, Spagnoli GC, Zajac P. CD40 ligand-expressing recombinant vaccinia virus promotes the generation of CD8+ central memory T cells. Eur J Immunol, 2016, 46: 420–31

Governa V, Trella E, Mele V, Tornillo L, Amicarella F, Cremonesi E, Muraro MG, Xu H, Droeser RA, Däster S, Bolli M, Rosso R, Oertli D, Eppenberger-Castori S, Terracciano L, Iezzi G, Spagnoli GC. The interplay between neutrophils and CD8+ T cells improves survival in human colorectal cancer. Clin Cancer Res, 2016, in press

Connection to Clinical Practice

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Translational science in surgical oncology
Our studies are conducted in close interaction with clinical partners and pathologists, facilitating the access to clinical specimens and the validation of working hypotheses emerging from experimental studies by using large databases from clinically annotated archival tissue specimens. Moreover, collaborations with "Tissue engineering" and "Cancer Immunotherapy" research labs have allowed the establishment of tridimensional cultures in perfused bioreactors and the development of xenograft technology in immunodeficient animals, respectively. A major role in this context is played by young surgeons in formation, working full time in the lab for one year stages and getting acquainted with basic research techniques while bringing in clinical experience and helping to define translational research targets. Within this frame, the generation of novel, highly immunogenic recombinant vaccinia virus is expanding our platform of reagents for cancer immunotherapy previously tested in clinical trials in patients with advanced melanoma. Considering the ongoing explosion of knowledge on the therapeutic impact of innovative biologicals addressing tumor-immune system interaction, it is easy to predict that these reagents might be successfully utilized in the future in cancer immunotherapy in combination with antibodies targeting "immunological checkpoints". On the other hand the validation of the prognostic relevance of the analysis of immune contexture in CRC has the potential to impact on surgical techniques, by suggesting the use of methods adapted to the ability of the immune system to control cancer progression, and thus decisively ameliorating quality of life of treated patients.

Cancer- and Immunobiology

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Lipid signaling in cancer and inflammation – pharmacological targeting and spatial regulation

In normal tissue, cell fate is controlled by surface receptors triggering signaling events at the inner leaflet of the plasma membrane. Here, phosphoinositide 3-kinases (PI3K) produces PIP3 to initiate a kinase cascade culminating in the activation of protein kinase B (PKB/Akt) and mammalian target of rapamycin (mTOR). PI3Ks controls cell growth, proliferation, survival and migration. In cancer cells, multiple inputs trigger a continuous activation of the PI3K/mTOR pathway. PI3Ks are therefore considered as valuable drug targets in oncology and inflammatory disease.

BKM120 is one of the clinically most advanced PI3K inhibitors (PI3Ki), is currently in phase III clinical trials and listed in more than 80 clinical trials in oncology. In the framework of PI3K and mTOR-targeting projects supported by the CTI, we have elucidated the mechanism of a BKM120-mediated cell cycle arrest (Bohnacker *et al.* 2015). Interestingly, BKM120 increased mitotic markers such as phospho-Histone H3 in a large cancer cell panel, suggesting that the drug acted by a mechanism that is distinct from other PI3K inhibitors (Fig. 1). Typically, PI3K inhibitors such as PQR309, and GDC0941 and GDC0980, arrest cancer cells in the G1/S cell cycle phase. BKM120 was found to interact with tubulin and PI3K. To further monitor the two inherent biological actions of BKM120 we investigated structurally related molecules and generated a potent BKM120-derived microtubule (MT) disruptor MTD147. This chemical split of PI3K and MT activities inherent to BKM120 allowed a functional profiling associating BKM120 with mitotic arrest and Histone H3 phosphorylation. Interestingly, the BKM120-induced mitotic arrest was detected below reported AUC0-24 levels currently used in clinical studies. This suggests that there is no valid therapeutic window for PI3K inhibition without interference with MT stability.

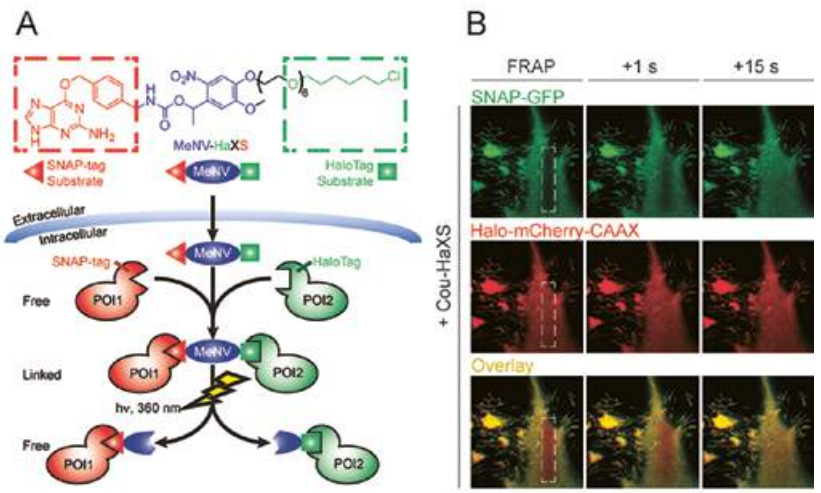

Fig. 1: A) Typically, PI3K inhibitors (PI3Ki) block the cell cycle in G1, and act cytostatic. When treated with a PI3Ki (here PQR309), cancer cells in mitosis can be rarely detected (mitotic marker: phospho-histone H3, pHistone H3). **B)** In contrast, BKM120 triggers a mitotic arrest with an accumulation of pHistone H3 positive cells, which accumulate in G2/M, condense nuclear DNA, and/or undergo apoptosis. At the same time cellular microtubules are disrupted.

A combination of chemical, cell biology and X-ray crystallography studies further elucidated why BKM120 blocked MT polymerization: BKM120 binds to the colchicine-binding pocket on β-tubulin. Binding depends on the orientation of BKM120's core pyrimidine ring. Moreover, biochemical, cellular and structural data suggests that the initially proposed orientation of BKM120 in PI3K is inverted. The in depth analysis of BKM120's activities mechanistically defined its dominant anti-tumor activity, and explains why PQR309 with a triazine core lacks the off-target effect on MT (Bohnacker *et al.* 2015).

The above structure function study also provided starting points for the production of very potent PQR309 follow-up molecules and mTOR kinase-selective inhibitors (which entered the drug development program of PIQUR Therapeutics (Beaufils *et al.* 2016; see sidebar), and potent microtubule polymerisation blockers with novel pharmaceutical characteristics. Altogether, the studies provide access to improved rational drug trials in drug combination studies, when combining PI3Ki and MT targeting drugs.

We have a long standing interest in localized lipid signalling, as this is crucial to PI3K signalling in cancer and inflammation. In the ESF-funded project "Tracking of Phosphoinositide Pools – Key Signaling Components in Cell Migration and Polarisation", we have initiated the generation of novel tools to control subcellular signalling enzyme localization. To be able to dock signalling enzymes to any location in a cell, we have produced so-called chemical-inducers of dimerization (CIDs). These molecules have two reactive groups that specifically form covalent bonds with proteins fused to a SNAP- or a HaloTag (Fig. 2). When one of the proteins is targeted to a cell organelle with a suitable anchor, a target protein can then be associated with this organelle by the addition of a cell permeable CID. To make this association reversible, photocleavable groups were incorporated into the CIDs. This resulted in molecules that allow the dynamic, time resolved and localized dissociation of target enzymes from selected membranes (Zimmermann *et al.* 2014, example in Fig. 2B).

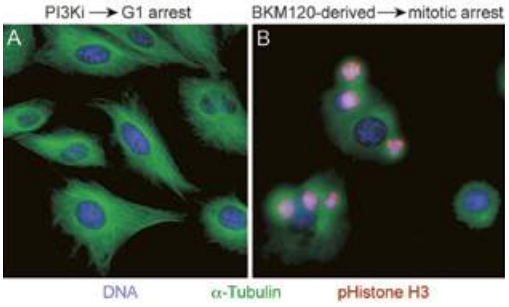

Fig. 2: A) The manipulation of the site of action of proteins of interest (POI) is important in lipid signaling research. The bi-functional chemical-inducer of dimerization (CID) MeNV-HaXS is cell penetrable, and reacts specifically with intracellular SNAP- and HaloTag-fused POIs. The covalent association of two tagged proteins can be reversed by illumination, where a 360 nm light pulse cleaves the MeNV group (Zimmermann *et al.* 2014). **B)** To improve the spectral properties of the CID, novel photocleavable molecules were produced: Cou-HaXS can be efficiently and rapidly cleaved at 405 nm, and is well suited for FRAP microscopy setups. The depicted TIRF images of HeLa cell plasma membrane demonstrate how a laser pulse within the highlighted section (broken lines) can dissociate a target protein (here SNAP-GFP) in a spatially controlled fashion from a membrane anchor (Halo-mCherry-CAAX). The lateral membrane diffusion of the CID-linked proteins is monitored by the recovery of the fluorescence intensities after the FRAP pulse.

Connection to Clinical Practice

PI3K/mTOR inhibitors – moving forward in clinical trials

Mainly due to its importance in cancer growth control, the phosphoinositide 3-kinase (PI3K)/mammalian target of rapamycin (mTOR) pathway is a field of intense efforts in pharmaceutical industry. PIQUR Therapeutics, a spin-off company of the University of Basel, has successfully completed phase I clinical trials with its lead compound PQR309. PQR309 is a potent, brain penetrable pan-PI3K inhibitor with moderate action on mTOR kinase activity. Phase II studies have been initialized in lymphomas and solid tumors. A first drug combination trial using PQR309 with eribulin, a microtubule-depolymerizing agent, has been initiated in a collaboration with Eisai in triple negative breast cancer patients (piqur.com; clinicaltrials.gov).

Support from the Swiss CTI spurred research at University of Basel to generate a number of novel lead compounds targeting PI3K and mTOR with defined selectivity and pharmacological profiles. Three compounds, a brain penetrant pan-PI3K/mTOR inhibitor, a highly potent pan-PI3K inhibitor and a very selective and brain accessible mTOR inhibitor, have already passed into preclinical validation, proof of principle, toxicity and preclinical safety studies. Besides the potential therapeutic value, the produced drug portfolio will provide opportunities to evaluate the biology of tumor drug resistance to PI3K/mTOR inhibition, and to develop tools for chemical genetics approaches.

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Smirnova NF, Gayral S, Pedros C, Loirand G, Vaillant N, Malet N, Kassem S, Calise D, Goudounèche D, Wymann MP, Hirsch E, Gadeau AP, Martinez LO, Saoudi A, Laffargue M. (2014) Targeting PI3Kg activity decreases vascular trauma-induced intimal hyperplasia through modulation of the Th1 response. J Exp Med 211, 1779–1792

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Cancer Immunology

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Immune modulation in cancer: implications for novel cancer therapies

Our group is a translational science laboratory in the field of cancer immunology/immunotherapy. We are applying basic research in immunocompetent murine models and tumor specimen from cancer patients. The direct connection to the medical oncology division allows translating preclinical discoveries into early clinical trials.

1. New combinatorial approaches to enhance cancer immunotherapy
A major goal is to develop rational combinatorial approaches that combine the unique ability of immunotherapy to mediate long-term responses and the significant benefits of cytotoxic and targeted anti-cancer therapies. There are now emerging experiences that the latter may turn immune-resistant tumors into tumors sensitive to immune-mediated killing by re-activating pathways within tumors responsible for its recognition and/or killing by immune effector cells. We discovered that microtubule-depolymerizing agents (MDA) such as maytansins and dolastatins are capable of inducing the full spectrum of maturational changes in dendritic cells (DCs), thereby potentiating the tumor-specific T cell response *in vivo*. These findings provided novel insights into the therapeutic activity of antibody drug conjugates including T-DM1 (antibody against HER2 coupled to MDA) in eliciting anti-tumor immunity in patients with early breast cancer and a HER2-expressing orthotopic tumor model. Importantly, the combined treatment of T-DM1 with checkpoint inhibitors (antibodies targeting the immune checkpoints CTLA-4 and PD1) induced the rejection of established tumors due to engagement of both innate and adaptive immune mechanisms. This finding led to the initiation of trials in HER2-positive breast cancer that use the combination of T-DM1 and checkpoint inhibitors targeting the PD1/PD-L1 axis.

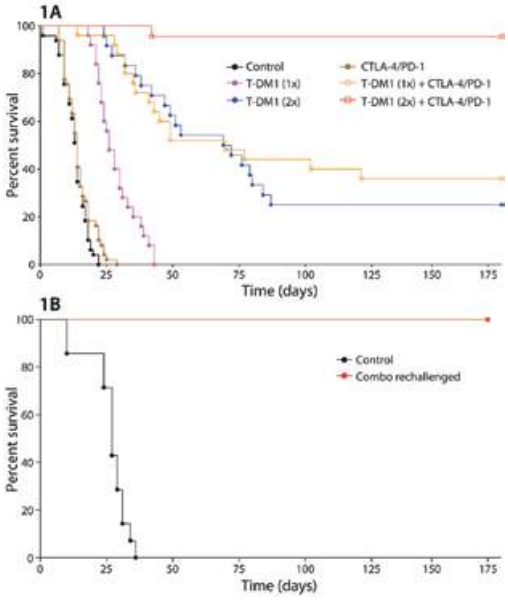

Fig. 1: (A)
Therapeutic response of the antibody drug conjugate T-DM1 and the combination with checkpoint inhibitors α-CTLA-4/-PD-1 in mice bearing tumors which overexpress human HER2 under the control of the murine mammary tumor virus promoter (Fo5) as a model of huHER2-overexpressing breast cancer.
(B) Mice which remained tumor-free after combination therapy and control mice were rechallenged in the contralateral mammary gland using cell suspensions from whole Fo5 tumor digests.

2. Mechanisms of immune cell dysfunction in cancer

We are interested in dissecting mechanisms underlying T cell dysfunction in cancer patients to improve cancer immunotherapy by identifying synergistic agents and optimizing patient selection. Recent work has elucidated the cumulative expression of inhibitory receptors as a hallmark of dysfunctional T cells and tumor progression, in particular in patients with non-small cell lung cancer. Of note, those inhibitory signals largely impact on the efficacy of treatment-induced immune activation and tumor cell killing. Moreover, changes of the glycosylation within the tumor microenvironment are investigated and recent work suggests that the cancer-associated upregulation of sialic acid-containing glycans can engage inhibitory receptors such as Siglecs and significantly inhibit the anti-tumor immune response.

3. Development of anti-cancer strategies in early clinical trials

Our clinical research focus lies on the investigation and development of treatment strategies, targets and delivery platforms in early trials in medical oncology. In collaboration with the Clinical Research Center (CCRC) at our division, we have programs ongoing to create a pipeline of agents that can move into the clinic. In translational projects, we aim at defining predictors of therapeutic responses and at understanding the mechanism of treatment responses and resistance. In addition, we define novel tumor antigens by analyzing the autoreactive antibody repertoire. The clinical programs include cancer vaccines, immune modulatory drugs, monoclonal antibodies, and nanoparticles such as immunoliposomes. In collaboration with the Department of Radiology and Nuclear Medicine, a program is centered on radiopeptides against peptide receptors. In addition, to optimally develop novel anti-cancer agents, in particular immunotherapeutics, *in vitro* organotypic assays are performed to study how these compounds modulate immune effector populations in freshly excised tumor tissue, thus closely mimicking the situation found in cancer patients. This program is performed in collaboration with the Department of Thoracic Surgery, Gynecology and Pathology.

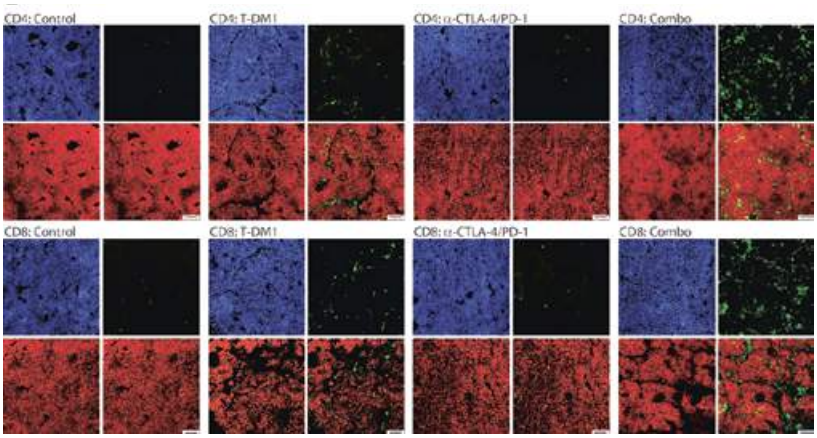

Fig. 2: Representative tumor sections treated as indicated were stained for CD4 (upper panel) and CD8 (lower panel) to assess changes of intratumoral immune cell subsets, (blue: DAPI; red: HER2, and green: CD4 or CD8, respectively) (scale bar = 100µm).

Selected Publications

Müller P, Kreuzaler M, Khan T, Thommen DS, Martin K, Glatz K, Savic S, Harbeck N, Nitz U, Gluz O, *et al.* (2015) Trastuzumab emtansine (T-DM1) renders HER2+ breast cancer highly susceptible to CTLA-4/PD-1 blockade. Sci. Transl. Med. 7, 315ra188

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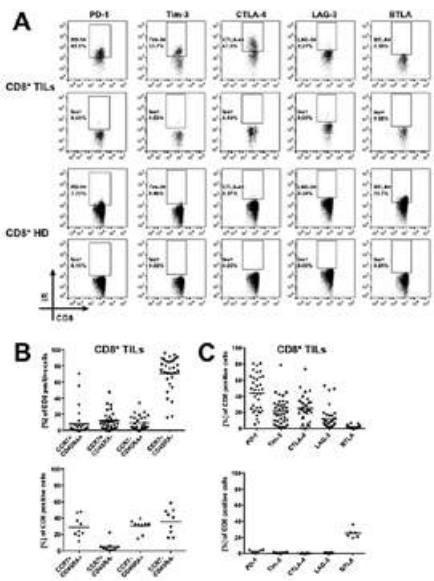

Fig. 3: Immune profile of tumor infiltrating CD8⁺ T cells (TILs) from NSCLC patients. **(A)** The expression of the inhibitory receptors PD-1, Tim-3, CTLA-4, LAG-3 and BTLA was determined by flow cytometry on tumor infiltrating CD8⁺ T cells from tumor digestions. **(B)** Distribution of naïve and memory T cell subsets, characterized by CCR7 and CD45RA, in CD8⁺ T cells from lung cancer specimens (TIL) or PBMCs from healthy donors (HD). **(C)** Expression of inhibitory receptors on tumor infiltrating CD8⁺ T cells.

Immunology

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During evolution an immune system has been generated for protection against life-threatening infections. However, the high versatility and complexity of the immune system also harbors the danger of developing various diseases like immunodeficiency's and autoimmune diseases. These diseases most often arise as a consequence of defects in the development of the immune system and/or as a consequence of aberrant immune reactions.

The focal area (FA) Immunology of the Department of Biomedicine currently comprises 27 research groups of which about half of the groups concentrate their research on more translational questions whereas the other half is tackling more basic questions. Recently the FA Immunology founded the University of Basel Immunology Community group (uBICO) with the goal to further strengthen the interactions between the immunology related research groups within Basel and to improve the training of PhD students of the FA Immunology. Currently members of uBICO are in charge of organizing the weekly immunomeetings, the annual retreat of the research groups within the FA Immunology, the PhD-club in which PhD students of the FA Immunology discuss their projects and the invitation of 3–4 distinguished guest speakers per year.

Of the more translational research groups within the FA Immunology, 6 concentrate their studies on a better understanding of the pathogenesis of various autoimmune diseases which include, SLE, Arthritis, Diabetes and MS. One group is studying primary immunodeficiencies and especially the link to the development of autoimmunity.

Viruses constitute a life-threatening challenge especially in individuals with primary or secondary immunodeficiencies. Four research groups within the Department focus their efforts on the immune system's early recognition of viral infection and ways by which an anti-viral response can be enhanced. Also fungal infections can have devastating effects especially in immuno compromised individuals. The factors that determine the risk of getting such an infection and the potential therapy of these patients is the research focus of one group within the Department.

For a long time it is known that vaccination is the method of choice to prevent infectious diseases. However, it has been recognized that not all individuals are able to mount a protective immuneresponse upon vaccination. One group within the FA Immunology is using a systems biology approach in order to improve vaccination strategies.

Several groups within the FA Immunology concentrate their research on various aspects of the development of the immune system. Dendritic cell subpopulations are key players in the initiation of various immune responses. One group is concentrating on the development of the different dendritic cell subsets with special emphasis on the role of transcription factors in these processes. Yet another group is focusing its research on the molecular mechanisms that guide lymphocyte development. The instructive and/or the permissive roles of cytokines and the involvement of various transcription factors in these developmental processes are the main research focuses of this group.

Regulatory T cells (Tregs) are T cells that can inhibit the function of other T cells and therefore are thought to be effective in the treatment of various diseases and could prevent organ rejection upon transplantation. Two groups within the FA Immunology study the development of Tregs and also address their potential therapeutic usage in different experimental models.

The thymus is the organ in which T cell development and education takes place. The thymus as an organ is rather complex and contains various epithelial subsets that play a crucial role in T cell development and education. One group of the FA Immunology is studying the development of these different epithelial subsets and the consequences of impairment in these developmental processes on the emerging T cell repertoires.

Innate lymphocytes (ILC's) show many similarities to T cells including the production of certain cytokines and the requirement of certain transcription factors for their development. However, unlike lymphocytes these cells do not express an antigen specific receptor. Over the years it has been well established that these ILC's play a crucial role in the proper functioning of the immune system. The requirement for one of these in lymph node organogenesis is perfect example of this. The research of one of the groups in the Department is focused on the development and functions of these ILC's.

T cells in our body play a crucial role in the protection against a wide range of pathogens. However, their differentiation and activation into the various effector functions is not yet fully understood. One group within the FA Immunology is using continuous time-lapse imaging combined with flow cytometry and gene expression profiling to address T cell activation in great detail.

In the last years it has been recognized that metabolic pathways play a crucial role in T cell function and longevity and that impairments in these pathways can underlie the development of various diseases. Up to now the signaling pathways that regulate the metabolic status in T cells are only poorly defined. The research focus of one of the groups within the FA Immunology is the unraveling of these signaling pathways with the major goal to identify new therapeutic strategies for patients with impairments in these.

Classical T cells recognize with their α/β TCR peptides bound to polymorphic MHC class I or class II molecules. However, over the years it became evident that a very significant number of non-classical T cells are also present in our body. These T cells recognize non-peptide antigens like lipids, glycolipids and small metabolites of microbial origin bound to MHC related molecules like CD1 and MR1. Two groups within the Department are studying the characteristics of these T cell in great detail and also address their potential role in diseases including infections. Taken together a wide variety of basic translational immunological research activities are ongoing in the Department. Moreover a network of laboratory based research with strong links to clinical medicine and other institutes ofthe University of Basel has been established.

Translational Immunology

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Giant Cell Arteritis – towards a better understanding of pathogenesis

Giant cell arteritis (GCA) is a primary vasculitis of unknown etiology. Disease manifests as an inflammatory syndrome and ischemic symptoms resulting from stenosis of inflamed arteries. There is strong evidence that T cells play an important role in disease induction and/or maintenance. The pathogenesis remains, however, unknown. One of the goals of our lab is to unravel the events leading to disease and more specifically the immunological target of the T cells.

Characterization of the immunological milieu and the T cell compartment in GCA
Using multiparameter flow cytometry immunophenotyping and multiplexed cytokine measurements we determined the immunological milieu in 42 study subjects (16 GCA patients at diagnosis, 13 disease controls and 13 non-inflammatory, age-matched controls). We identified IL-6 as the main cytokine differentiating between GCA and controls (Fig. 1). Similarly, the IL-8 was higher in GCA, while the other cytokines showed no significant difference to controls. This finding nicely fits the clinical data showing good response of treatment with an anti-IL6 receptor antagonist. Using multi-color flow cytometry we confirmed that T regulatory cells seemed to be reduced in GCA compared to controls. Th1 T cells were comparable between the groups, while high IL-17-producing CD4+ and CD8+ T cells were more frequent in GCA compared to all controls (Fig. 2). However, the majority of GCA patients had normal frequencies of Th17 T cells, suggesting that distinct patient subgroups might exist. As part of the analysis between the clinical presentation and immunological data we are currently investigating this.

Determination of the T cell repertoire in GCA
As next steps, we aim to determine the T cell receptor (TCR) repertoire in patients with GCA. Specifically, we want to test whether the T cells in the inflamed tissue of GCA patients or self-reactive T cells in the peripheral blood are oligoclonally expanded. This information can be used to screen for the immunological target of the T cells.

The role of autoantibodies targeting 14-3-3 protein isoforms in GCA
A recent study suggested that antibodies against isoforms of the so-called 14-3-3 protein (an intracellular regulatory protein) may be useful as biomarkers in large-vessel avsculitis (LVV), i.e. GCA and Takayasu's arteritis. This study was done in patients with aortic aneurysm due to LVV, i.e. those with a late complication of vasculitis. Here, we performed an analysis to assess the presence of these Autoantibodies 'at GCA diagnosis'. If present they would have the high potential as biomarkers/autoantibodies for the diagnosis and monitoring of treatment effects as in other autoimmune diseases (e.g. systemic lupus, ANCA vasculitis). To test this, antibodies against three isoforms of 14-3-3 (γ, ε, and ζ) were measured in 51 LVV patients, and 42 controls (including non-inflammatory and inflammatory diseases), using an multiplexed bead-based immunoassay and immunoprecipitation assays. The positive threshold was defined based on values found in young healthy controls. Anti-14-3-3 IgG antibodies were compared between GCA patients and controls to assess their diagnostic performance as a biomarker. Antibodies against all three 14-3-3 isoforms were detected in GCA patients as well as in age-matched inflammatory and non-inflammatory controls. Amongst LVV patients, detection of antibodies targeting 14-3-3 ε and ζ was associated with more severe disease, specifically stroke or aortitis. Thus, we could conclude that detection of antibodies against 14-3-3 proteins at the time of GCA diagnosis is not disease-specific. Their

presence at high levels in LVV with stroke, aortitis and – in a previous study – with aneurysm formation may indicate their value as potential biomarkers for extensive large vessel inflammation. The relevance of 14-3-3 antibodies in non-LVV patients needs to be tested in larger cohorts.

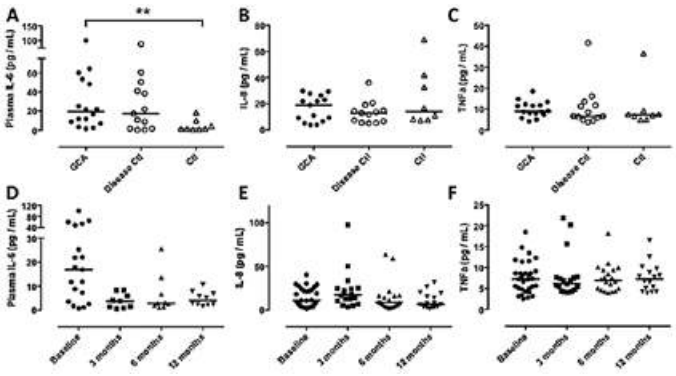

Fig. 1: Cytokine profiles in GCA and controls. (A–C) Mesoscale measurements for three representative proinflammatory cytokines – IL-6, Il-8 and Il-1 – are displayed. IL-6 levels were high in GCA and inflammatory controls. The latter includes many patients with polymyalgia rheumatica (PMR) a disease sharing many features with GCA, including the good response to anti-IL6 therapy (Tocilizumab). **(D–E)** Longitudinal measurements indicate that upon therapy IL6 levels drop dramatically. Kruskal-Wallis test with Dunn's correction was applied. *P < 0.05.

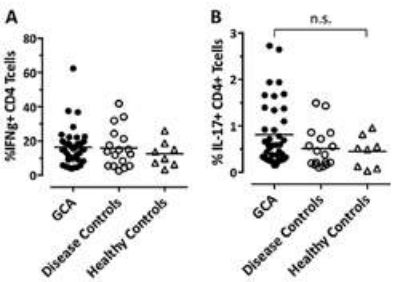

Fig. 2: A subset of GCA patients is characterized by high Th17 T cells. The percentage of IL-17-producing CD4+ T cells in lymphocytes of GCA patients, disease controls and healthy controls was determined by flow cytometry using PMA stimulation assays. Data is expressed as % positive for the respective cytokine amongst CD4 T cells. n = 42 (23 GCA, 12 disease controls, 7 healthy controls)

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Bigler MB, Egli SB, Hysek CM, Hoenger G, Schmied L, Baldin FS, Marquardsen FA, Recher M, Liechti ME, Hess C, *et al.* (2015). Stress-Induced *In Vivo* Recruitment of Human Cytotoxic Natural Killer Cells Favors Subsets with Distinct Receptor Profiles and Associates with Increased Epinephrine Levels. PLoS One 10, e0145635

Berger CT, Greiff V, John S, Koenig KF, Bigler MB, Recher M, Hess C and Daikeler T (2015). Risk factors for pneumocystis pneumonia in giant cell arteritis: a single-centre cohort study. Clin Exp Rheumatol 33, S-122–125

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Connection to Clinical Practice

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The Basler Giant Cell Arteritis Cohort

Giant cell arteritis (GCA) is the most prevalent of the primary vasculitis syndromes with an increasing disease incidence. Patients typically present with constitutional symptoms, headache, and a systemic inflammatory syndrome. To date therapy of GCA is based largely on steroids, and guided by parameters reflecting disease activity only partially, as indicated by recent imaging-studies. Furthermore, intensity and duration of steroid therapy remain a matter of debate, and no consensus exists in defining remission. Both GCA itself and the steroid based therapy are associated with significant morbidity. Improving diagnostic accuracy and monitoring of disease activity thus would be of great importance. To study these clinical problems, we established a prospective interdisciplinary cohort of patients with GCA. Relevant clinical data, laboratory parameters, serum and peripheral blood mononuclear cells from all patients are collected at longitudinal time-points. Vascular disease activity is assessed using new technologies such as color-coded duplex ultrasound and positron emission tomography. Thereby we aim at integrating clinical data, imaging studies, and extended immunological and histomorphological assessments for a more detailed understanding of the immunopathogenesis of GCA. This may help to (i) further develop precise, ideally non-invasive, tools to diagnose and monitor disease activity, and (ii) generate strategies towards interfering with specific pathways associated with disease activity and/or complications.

Translational Diabetes

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Immune cell activation in metabolic disease

One of our research interests is the role of macrophage polarization in metabolic disease such as type 2 diabetes and obesity. Polarization of macrophages is characterized by their inflammatory activation and metabolism status. Gaining knowledge about the polarization of macrophages is crucial for a better understanding of the pathogenesis of metabolic disease. Deliberate modulation of macrophage polarization could help to improve glycemic control. Selectively targeting increased glycolysis has already been suggested for anti-cancer therapy. However, it has not yet been studied in metabolic diseases. Our aim is to target macrophage polarization and thereby improve glycemic control.

As a potential pharmacologic agent to target macrophage polarization, we assess the effect of the tyrosine kinase inhibitor (TKI) imatinib. Imatinib is approved for the treatment of multiple cancers, most notably for chronic myelogenous leukemia (CML). The primary target of TKIs is the constitutively active tyrosine kinase fusion protein created by chromosome translocation in CML. Imatinib has been shown to suppress aerobic glycolysis in leukemia cells and to drive polarization of tumor-associated macrophages from a pro- to anti-inflammatory phenotype. Interestingly, improvement in glycemic control has been described as a concomitant phenomenon in diabetic patients treated with TKIs. Thus, based on these known effects of TKIs on immune cells and glycemia, we assess the effects of imatinib on macrophage polarization and whether such changes go along with improved glycemic control. The aim is to find ways to deliberately modulate the level of immune cell polarization and subsequently glycemic control.

Moreover, we are also interested in the gastrointestinal immune system's role in mediating systemic inflammation in metabolic disease. Specifically, we are aiming to decipher the interplay between food intake, gut microbiota, the intestinal immune system, and systemic inflammation. Unhealthy "dysbalances" between these players are associated with a broad spectrum of diseases including metabolic disease. Intestinal immune cells could serve as crucial "sensors" of environmental factors (food intake, environmental toxins or altered microbiota) for the host's immune system. We are interested in how intestinal macrophages respond to different environmental stressors. Our aim is to better understand initial changes in immune cell activation that eventually mediate and maintain enhanced systemic inflammation in metabolic disease.

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Experimental Immunology

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Adaptive and innate T cells specific for non-peptidic antigens: the diseases' perspective

Antigen recognition is a central event in immune response and the immune system has evolved a series of receptors, which recognize a variety of antigens and activate specific immune cells. T lymphocytes express membrane-bound T cell receptors (TCR) that recognize complexes composed of antigen-presenting molecules and antigens. In addition to small peptides, T cells may also recognize lipids, glycolipids, and small metabolites of endogenous or microbial origin. Our studies in humans revealed that these latter T cells are as abundant as peptide-specific ones. Our goal is to understand the role of non-peptide-specific T cells in the immune response, and their participation in diseases.

Our studies cover the following three main areas. The first one is lipid-specific T cell immunity. Our aim is understanding the mechanisms leading to lipid recognition by T cells, how lipid antigens interact with the lipid-presenting CD1 molecules, and how lipid-specific T cells participate in the recognition of tumor cells (in human cancer), of mycobacteria-infected cells (in tuberculosis) and of self lipids (in autoimmune diseases). We have investigated the mechanisms how complex lipids are processed by lipases and hydrolases, how the lipid antigens are transported within the antigen-presenting cells, how they are loaded on CD1 molecules. These studies led to the establishment of novel anti-bacterial vaccines taking advantage of lipid-specific T cells providing help to B cells secreting sugar-specific antibodies. We have also identified novel tumor-associated lipid antigens, which will be further exploited in leukaemia immunotherapy.

The second type of studies investigate the biology of TCR γδ cells. We identified butyrophilin 3A1 as the relevant molecule for the activation of human T cells expressing the TCR Vγ9-Vδ2 heterodimers by microbial and self-metabolites. These metabolites accumulate in some tumor cells and specifically stimulate TCR Vγ9-Vδ2 cells. We established a human TCR Vγ9-Vδ2 transgenic mouse model that is being utilized to explore the anti-tumor function of this T cell population.

The third type of studies focus on T cells restricted to the MHC-class-I-related molecule, MR1. These T cells are stimulated by metabolites generated in the vitamin B2 pathway and are called mucosal-associated T (MAIT) cells as they preferentially localize within mucosal tissues, liver and skin. We found that the TCR gene repertoire of these cells is remarkably oligoclonal both in peripheral blood and liver, inferring preferential stimulation of selected T cell clones *in vivo*. Unique aminoacids were detected in the CDR3 regions of skin-derived MR1-restricted T cells, possibly due to selective expansion following stimulation with metabolites produced by the skin-resident microflora. Biochemical purification revealed a complex array of stimulatory antigens, including vitamin B2-unrelated ones.

In order to understand the functional role of MR1-restricted T cells, systematic and sequential studies are ongoing. First, by single cell transcriptomics analyses of MAIT cells we discovered functionally different cell populations, whose roles in liver and gut diseases are being investigated. Secondly, by multidimensional flow-cytometry we identified the phenotypic correlates of these distinct populations. To this aim, panels of monoclonal antibodies have been optimized to allow the characterization of T cells within human healthy and diseased tissues. Thirdly, individual cell populations are being investigated after sorting, cloning and *in vitro* activation. These studies are revealing how tissue-resident MR1-restricted T cells specialize in their functions and participate in disease evolution.

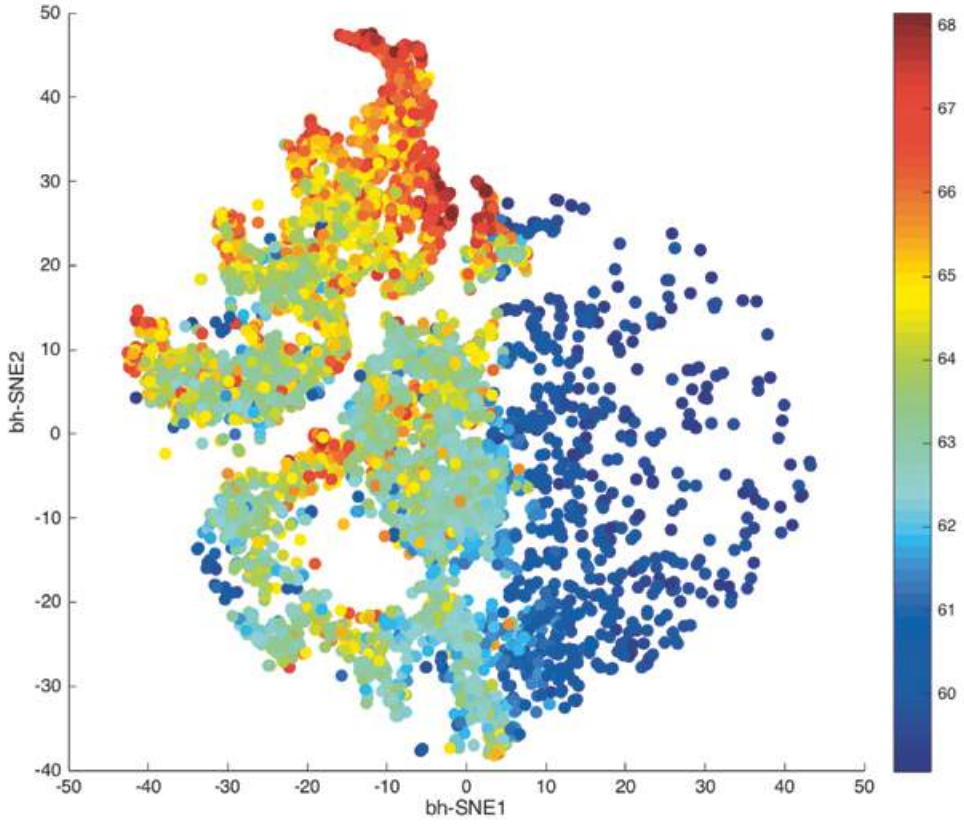

Fig. 1: Populations of MAIT cells identified by multidimensional flow cytometry. Differential expression of 14 surface markers upon stimulation with microbial metabolites. Colors indicate antigen responsiveness: non-reacting cells (blue) highly reacting cells (red).

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Diabetes Research

Islet inflammation in physiology, obesity and type 2 diabetes

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Research Summary: Our research focuses on the mechanisms and therapy of insulin production by the pancreatic islets in physilogy and in obesity associated type 2 diabetes. In previous studies we demonstrated that the metabolic stress evoked by high glucose and saturated fatty acids (contained in animal fat) may induce death of the insulin producing beta-cells of the islets. Subsequently we identified interleukin-1 beta as a key mediator of these deleterious effects and showed that it is produce by human beta-cells in type 2 diabetes. We published several additional studies supporting the concept that this mechanism leads to an inflammatory process and underlies the failure to produce sufficient amount of insulin in type 2 diabetes. On the basis of this we initiated clinical trials in patients with type 2 diabetes that vindicates this hypothesis and opens the way for a causative treatment. Furthermore we identified a new endocrine loop by showing that elevated IL-6 mediates a cross talk between insulin sensitive tissues, L cells and pancreatic islets to adapt to changes in insulin. Finally, we uncovered the first monogenic form of type 1 diabetes. This research has contributed to the concept that the innate immune system is part of the regulation of metabolism.
Our ongoing studies aim at understanding the physiological role of the immune system in metabolism. The general objective is to uncover mechanisms on how the innate immune system contributes to the adaption of pancreatic islet function under various physiological (postprandial and pregnancy) and stressful (obesity and diabetes) situations with a focus on macrophages derived IL-1beta in the postprandial stimulation of insulin; Beta-cell and placenta produced IL-1beta and IL-1Ra, and of alpha cell derived GLP-1 in physiology and insulitis; IL-33 induced ILC2 stimulated insulin secretion.

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Selected Publications

Maedler K, Sergeev P, Ris F, Oberholzer J, Joller-Jemelka HI, Spinas GA, Kaiser N, Halban PA, and Donath MY. 2002 Glucose-induced β-cell production of interleukin-1β contributes to glucotoxicity in human pancreatic islets. J. Clin. Invest. 110:851–860

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Applied Microbiology Research

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Towards a system understanding of immune responses, personalized vaccination strategies, and pathogen transmission

Hosts and pathogens share complex interactions across scales from molecules to populations. The Applied Microbiology Research group aims to understand these various levels of interactions by the identification of key factors involved using a systems biological approach (Fig. 1). We want to translate our understanding of the complex host-pathogen interactions into clinical applications, such as novel adjuvant development targeting specific signaling pathways or preventive measurements on a population level. As a main model to explore the host-pathogen interaction, we use influenza vaccination and epidemic transmission.

Vaccine response to influenza

Influenza infection is associated with significant morbidity and mortality in immunosuppressed hosts, such as patients after allogeneic stem cell transplantation. Although vaccination is *the* preventive strategy, many vaccinated patients fail to mount a protective humoral immunity. Along with transplant related factors such as time after transplantation, immune reconstitution and graft-versus-host diseases, the genetic background of each patient also plays a crucial role in modulating and building seroprotection against influenza (and every other pathogen).
Currently, we are exploring the impact of genetic polymorphisms (single nucleotide polymorphisms, SNPs) on the interferon signaling pathway to influenza-specific humoral vaccine responses in a multi-center vaccine trial. SNPs in the Interferon (IFN)-lambda pathway may influence the way B-cells encounter vaccine antigens. Alternative variants of IFN-lambda (IFN lambda 1-3) show different binding affinities to the receptor (Fig. 2A). This is mainly dependent on a few amino acid differences at the binding interaction site (Fig. 2B). B-cells show a significant response to members of the IFN-lambda family (IFNL1-3) (Fig. 2C). Influenza vaccine recipients might therefore, based on their genetic background in the Interferon lambda genes show important variability in the vaccine responsiveness. The immunological data is computationally modeled in collaboration with the D-BSSE (ETHZ, Prof. Stelling). This may allow the development of personalized vaccine strategies.

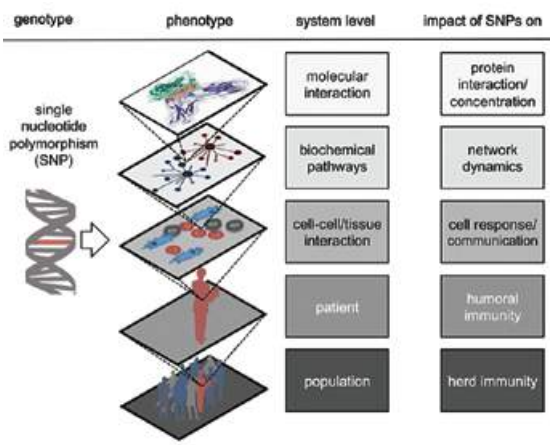


Fig. 1

Fig. 2A

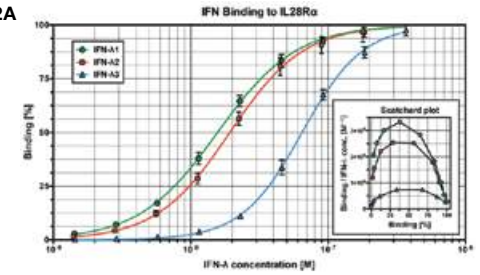


Fig. 2B

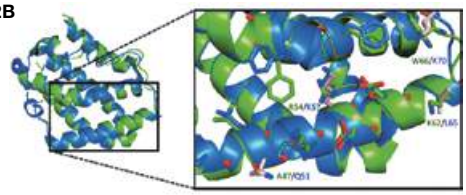


Fig. 2C

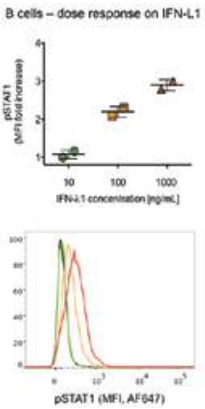


Fig. 3A

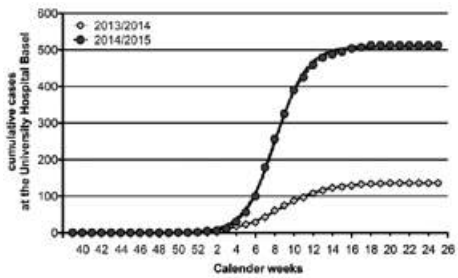
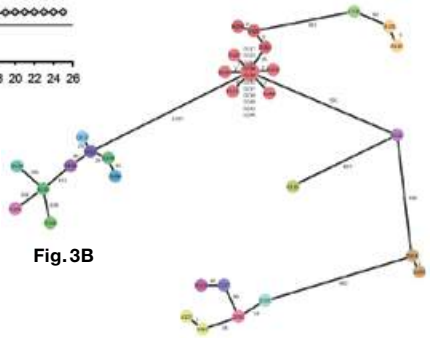


Fig. 3B



Influenza transmission in an urban population

Influenza transmission is highly complex and dependent on multiple factors such as population density, age distributions, and individual and herd immunity. Although the severity of a flu season can be described with the basic reproduction number (R0, Fig. 3A), which serves as a surrogate for transmission efficacy, it remains unclear where and in what context influenza transmission actually happens. In an interdisciplinary and inter-institutional collaboration with the Human Geography and Center for Primary Health Care (both University of Basel), Infectious Diseases and Hospital Epidemiology and Emergency Medicine (both University Hospital Basel), Pediatric Infectious Diseases and Emergency Medicine (both Children's Hospital University of Basel) and the Computational Evolution Research Group (Department of Biosystems Science and Engineering, ETH Zurich), we aim to explore the influenza transmission in the City of Basel.

Using whole genome sequencing, a technology which has been established for a broad series of pathogens (Fig. 3B), we will determine phylogenetic relationships of influenza samples and transmission pathways. The whole genome data will be used to develop a model to identify where and in what context influenza transmission is most efficient. Besides influenza, such models can be adapted to other infectious diseases. This will provide important information to identify and develop novel preventive counter measurements.

Connection to Clinical Practice

Influenza vaccination and transmission

Immunosuppressed patients, such as stem cell transplant recipients, are at a highest risk for complications during influenza infection. Vaccination is the key element in the preventive strategy. We aim to understand the clinical and immunological factors associated with vaccine failure in this patient population and to identify clinical and immunological markers associated with vaccine outcomes after transplantation. This should help to guide a vaccine protocol and to improve the overall outcome.

We have conducted a multi-center vaccine trial (Basel, Zurich, Berne, Lucerne, Aarau, and Ticino), building a biobank including immune cells, serum and DNA at various time-points post-vaccine to further explore the vaccine response at multiple levels. We are currently developing computational models to identify high-risk patients and predict vaccine outcomes. Such models could also be adapted for other pathogens with available vaccines such as *S. pneumonia* and *N. meningitidis.*

Selected Publications

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Liver Immunology

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Characterization of mucosal-associated invariant T cells in liver diseases

Accumulating evidence suggests that the gut microbiome (i.e. gut resident bacteria) is involved in the pathogenesis of various liver diseases. Interestingly, a specialized T cell subset belonging to the family of innate-like lymphocytes is highly abundant both in the gut mucosa and the liver. These mucosal-associated invariant T (MAIT) cells respond to bacterial metabolites produced in the vitamin B2 (riboflavin) synthesis pathway, what places them at a central position in the immunological gut-liver axis. Antigen presentation to MAIT cells involves the highly conserved MHC-related protein MR1. Since these MR1-restricted T cells were identified in the liver only recently, little is known about their function in healthy and diseased liver.
Our aim is to elucidate the role of MAIT cells in selected liver diseases, including autoimmune liver diseases, steatohepatitis and viral hepatitis, and in the fibrogenic response in the liver. Our studies address a largely unexplored area of human liver physiology and pathology. Due to their location and high abundance in the liver, and their responsiveness to bacterial products and various cytokines, we hypothesize that liver-resident MAIT cells play a role in the pathogenesis of liver diseases and that changes in MAIT abundance, activation status, and cytokine expression profile influence disease development and outcome.

Our study has three main goals:

1. To characterize liver-resident MAIT cells using patient-derived liver biopsies and blood samples. Using both cellular and molecular approaches, we are determining MAIT cell location and abundance in the liver and blood in different pathological conditions, and analyzing their functional and transcriptional profiles.

2. To determine how the gut microbiome influences MAIT cells present in the liver, gut, and blood. By analyzing tissue and stool samples from patients with liver diseases, we aim at establishing whether changes in bacterial composition parallel changes in the functionality of MAIT cells.

3. To identify and characterize interactions of MAIT cells within the liver environment. In cell culture experiments, performed with distinct purified subpopulations of primary human liver cells, we are defining the cellular and cytokine milieu contributing to MAIT cell activation.

The anticipated outcome of our experiments is identification of disease-specific changes and patterns that will allow us to draw conclusions about how MAIT cells influence liver physiology and pathology.

Connection to Clinical Practice

Most liver diseases are characterized by an inflammatory response in the liver, driven by numerous triggering conditions, such as exposure to alcohol, excess fat, medication, infection or autoimmunity. A prolonged inflammatory state leads to progressive fibrosis that can result in liver cirrhosis associated with serious complications including loss of liver function or development of hepatocellular carcinoma. To date little is known about the function of MAIT cells in healthy and diseased liver and very few studies have addressed a role of these cells in human liver tissue and using primary human material. Our projects are being performed in close collaboration with the Laboratory of Experimental Immunology, headed by Prof. Gennaro De Libero, and the Department of Gastroenterology and Hepatology at the University Hospital in Basel, headed by Prof. Markus Heim.

Developmental Immunology

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Innate immune regulation and lymphoid tissue induction

The innate lymphoid cell (ILC) family has emerged as the first line of defense against various pathogens. In addition, ILCs regulate tissue homeostasis and repair through the release of cytokines. Based on effector cytokines and transcriptional regulation they can be divided into three populations: group 1 ILCs, group 2 ILCs and group 3 ILCs including lymphoid tissue inducer (LTi) cells (Fig. 1). Studies in mouse and men clearly demonstrate that each subset has defined immune functions that depend on the tissue localization, type of activation and disease status. The molecular mechanisms underlying the tissue-specific regulation of ILCs are not fully understood. In particular, the exposure to environmental signals from commensals and nutrition factors has an effect on local expansion and activation of ILC subsets. Our work focuses on understanding the developmental requirements for generating ILCs and for regulating their activation and immune response in situ.

One major topic of investigation is assessing the molecular pathways that coordinate ILC development and tissue homeostasis. In recent years we have identified cytokines that act as master regulators of ILC and lymphoid tissue development. Notably, IL-7 and Flt3L are key cytokines in promoting the development of ILCs from fetal liver and common lymphoid progenitors in the bone marrow. Deletion of flt3l severely reduces the number of LTi cells in the neonatal intestine, resulting in impaired development of Peyer's patches, whereas IL-7 is critical for lymph node development (Fig. 2). In adults, both Flt3L and IL-7 regulate the generation of ILCs, although both cytokines operate at different developmental stages of progenitor cells. The complex network of cytokines in various tissues and stages of development may explain how ILCs can further mature in peripheral tissues.

A second focus of our research is the regulation of adaptive immune responses by group 3 ILCs. ILC3s respond to various "danger signals" with a specific profile of cytokines, which contribute to T cell polarization and activation of innate immune

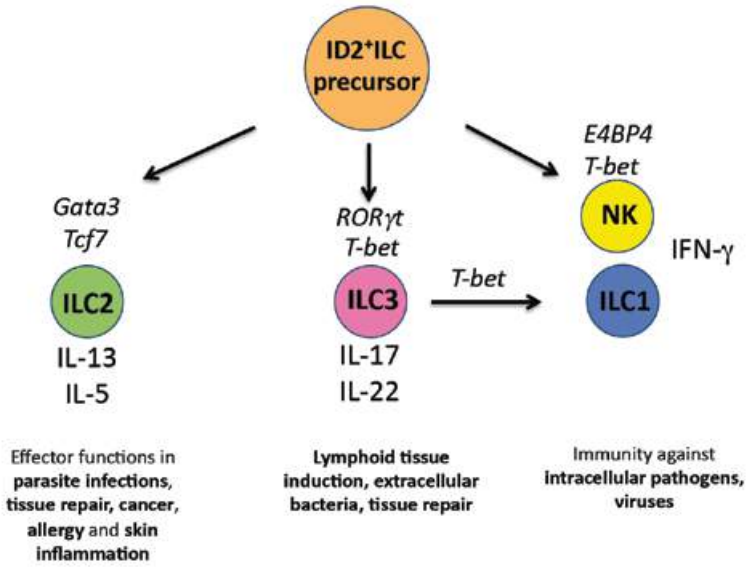

Fig. 1: Innate lymphoid cell (subsets): transcriptional regulation, cytokine release and effector functions.

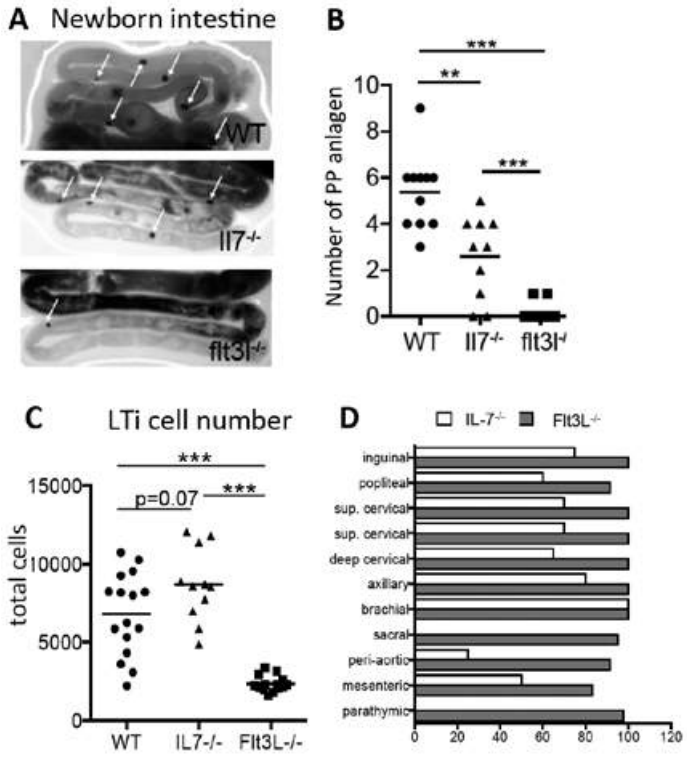

Fig. 2: (A) and (B) Peyer's patch anlagen in the small intestine of day 0.5-old *WT, Il7*$^{-/-}$ and *flt3l*$^{-/-}$ mice visualized by VCAM-1 whole-mount staining. Representative pictures of VCAM-1^{+} spots (indicated by arrows) are shown. (C) Total number of LTi cells in the small intestine of day 0.5-old *WT, Il7*$^{-/-}$ and *flt3l*$^{-/-}$ mice. (D) Presence of LNs in adult *Il7*$^{-/-}$ and *flt3l*$^{-/-}$ mice compared with *WT* set as 100 % (n = 6–10).

cells. We have reported that ILC3s have the capacity to internalize antigens (Fig. 3) and to present MHC-peptide ligands to T cells. Interestingly, according to their tissue of origin, splenic and intestinal (lamina propria (LP)) ILC3s have opposite antigen-presenting functions. Comparable to dendritic cells, splenic ILC3s are able to undergo a process of maturation (activation) upon IL-1β stimulation and induce antigen specific T-cell proliferation. By contrast, LP ILC3s lack co-stimulatory molecules after IL-1β stimulation and induce only very weak T-cell proliferation. In addition, a role in the negative regulation of T-cell responses against the gut microbiota was assigned to LP ILC3s. Hence, differences in the antigen-presenting function of splenic and LP ILC3s are that the former could be considered as immunogenic and the latter tolerogenic. The genetic analysis of both subsets has demonstrated subset-specific transcriptional profiles under steady-state and inflammatory conditions. We could further demonstrate that ILC-T cell interactions are bi-directional and that Ag-activated T cells provide feedback signals for activation of ILCs. Our current interest is to better understand the molecular pathways, which shape the tissue-specific ILC subset repertoire and immune function in the presence and absence of an adaptive immune system.

A third topic of interest is to understand the regulation of early and late effector cytokine responses of ILCs in health and disease. The tissue protective role of IL-22 produced by ILC3s has been well documented. On the other hand, ILC3s can convert into pro-inflammatory IFNγ-producing ILC1s (Fig. 1) thus demonstrating a high degree of plasticity. Using gene-targeting strategies we study key pathways promoting pro-inflammatory ILC responses in intestinal inflammation and cancer. Together, we use systematic molecular and functional analysis of ILC subsets in genetically modified mouse models and models for human inflammatory diseases to gain insights into the role of ILCs in chronic inflammation and tumor diseases.

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Baerenwaldt A, von Burg N, Kreuzaler, M, Sitte, S, Horvath E, Peter A, Voehringer D, Rolink AG, Finke D. (2016) Flt3 Ligand regulates the development of innate lymphoid cells in fetal and adult mice. J. Immunol., 196 (6):2561–71

Von Burg N, Turchinovich G, Finke, D. (2015) Maintenance of immune homeostasis through ILC/T cell interactions. Frontiers in Immunology, 6, 416

Schmaler M, Broggi MA, Lagarde N, Stöcklin BF, King CG, Finke D, Rossi, SW. (2015) IL-7R signalling in regulatory T cells maintains peripheral and allograft tolerance in mice. PNAS, 112 (43):13330–5

Brasseit J, Althaus-Steiner E, Faderl M, Dickgreber N, Saurer L, Genitsch V, Dolowschiak T, Li H, Finke D, Hardt WD, McCoy KD, Macpherson AJ, Corazza N, Noti M, Mueller, C. (2015) CD4 T cells are required for both development and maintence of disease in a new mouse model of reversible colitis. Mucosal Immunology, 9, 689–701

Von Burg N, Chappaz S, Baerenwaldt A, Horvath E, Dasgupta SB, Ashok D, Pieters J, Tacchini-Cottier F, Rolink H, Acha-Orbea H, Finke D. (2014) Activated group 3 innate lymphoid cells promote T-cell-mediated immune responses. PNAS, 111(35): 12835–40

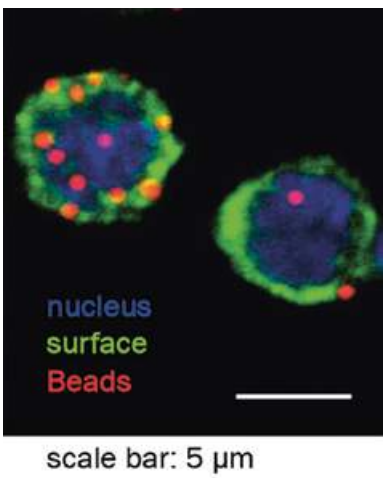

Fig. 3: Representative immunofluorescence image of red fluorescent latex bead uptake by sort-purified *in vitro* generated ILC3s.

Integrative Biology

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Feral pigeons and fish mobbing a worm

A Science communication project: The "Basler Taubenaktion 2016"

The food basis is the ecological minimum factor for the feral pigeon *(Columba livia)* in the urban ecosystem. We could show, that a food reduction leads to a reduction of breeding success and finally to a decrease of the population (Stock & Haag-Wackernagel 2016). Pigeon feeding enlarges the ecological capacity. Overpopulation as consequence of a large food basis is linked with several problems summarized as the "pigeon problem". This includes stress for the pigeons as well as increased risk of disease and parasite infestation. For the city dweller, the pigeon problem mainly consists of the risk of diseases and parasite transmissions from feral pigeons to humans, as well as structural damages due to fouling with pigeon feces.

In a joint project of our research group with our University (department events), the Cantonal Police Basel, Basel City Gardeners, Medical Services of the Department of Health and the Animal Protection Society Basel and Agglomeration, we implemented the results of our research in the "Pigeon Action of Basel 2016". With a multilingual poster, a flyer and a brochure we tried to convince the public not to feed pigeons. Our pigeon action has been well perceived by the communication media and we hope that the impact of our public education campaign will contribute to the solution of the pigeon problem.

Host finding of the pigeon tick *Argas reflexus*

The medically and veterinary important feral pigeon tick *Argas reflexus* usually feeds on pigeons, but if its natural hosts are not available, it also enters dwellings to bite humans that can possibly react with severe allergic reactions. *Argas reflexus* is ecologically extremely successful as a result of some outstanding morphological, physiological, and ethological features. Yet, it was still unknown how the pigeon tick finds its hosts. In our study, different host stimuli such as living nestlings as well as begging calls, body heat, smell, host breath and tick feces, were tested under controlled laboratory conditions. Of all stimuli tested, heat played an

Fig. 1: With the Basler Taubenaktion we implemented the results of our research into public education.

important role in host-finding. To confirm our laboratory results under natural conditions, the heat stimulus was tested within a pigeon loft. Therefore, we set up electronically heated modules of the size and body temperature of pigeons and monitored the reaction of the ticks. The results showed that *A. reflexus* is able to find a host over short distances of only a few centimeters. Furthermore, it finds its host by random movements and recognizes a host only right before direct contact is made. With our findings, we hope to contribute to the control of *A. reflexus* in infested apartments, both to diagnose an infestation and to perform a success monitoring after disinfestation.

Fig. 2: The pigeon tick *Argas reflexus* finds its hosts by their body heat.

Mobbing strategies of a fish against a sessile annelid predator

When searching for food, foraging fishes expose themselves to hidden predators. The strategies that maximize the survival of foraging fishes are not well understood. In an underwater wildlife study performed in the Lembeh Strait (Sulawesi, Indonesia) we describe a novel type of mobbing behaviour displayed by foraging Peters' monocle bream (*Scolopsis affinis*). The fish directs sharp water jets towards the hidden sessile annelid predator *Eunice aphroditois* (Bobbit worm). We recognized two different behavioural roles for mobbers (i.e., initiator and subsequent participants). The first individual to exhibit behaviour indicating the discovery of the Bobbit directed more water jets, absolutely and per time unit, than the subsequent individuals that joined the mobbing. We found evidence that the mobbing impacted the behaviour of the Bobbit, e.g., by inducing retraction. *S. affinis* individuals either mob alone or form mobbing groups. We suppose that this behaviour may provide social benefits for its conspecifics and in securing foraging territories for *S. affinis.* Our results reveal a sophisticated and complex behavioural strategy to protect against a hidden predator.

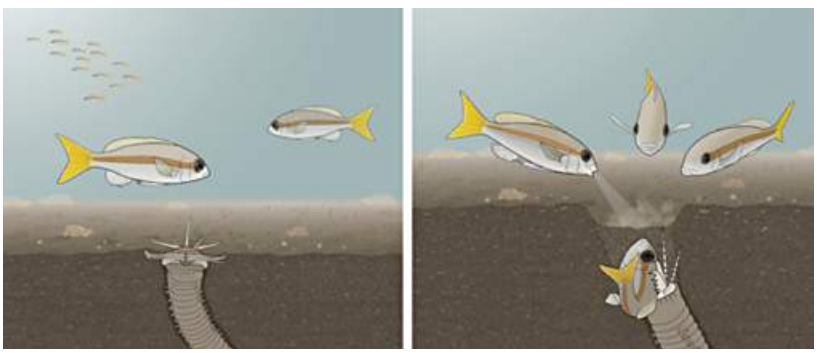

Fig. 3: Predation of *Scolopsis affinis* and subsequent mobbing of the Bobbit worm.
(a) The ambushing Bobbit is covered with sand and lures its prey with the protruding antennae; the jaws are under tension like an armed spring trap. (b) The Bobbit grasps and tears its prey into its burrow, and sand slips into the pit. Other *S. affinis* individuals approach and mob the Bobbit by blowing water jets into the pit.

Selected Publications

Stock, B., Haag-Wackernagel, D. (2014). Effectiveness of Gel Repellents on Feral Pigeons. Animals 4: 1–15.

Schreiber, T., Kamphausen, L., Haag-Wackernagel, D. (2015). Umwelteinflüsse und Gesundheitszustand bei Strassentauben *(Columba livia).* Effects of the environment on health of feral pigeons *(Columba livia).* Berl Munch Tierärztl Wochenschri 128 (1/2): 10–24.

Stock, B., Haag-Wackernagel, D. (2016). Food shortage affects reproduction of Feral Pigeons *Columba livia* at rearing of nestlings. Ibis. doi: 10.1111/ibi.12385.

Boxler, B., Odermatt, P., Haag-Wackernagel, D. (2016). Host finding of the pigeon tick *Argas reflexus.* Medical and Veterinary Entomology 30, 193–199.

Lachat, J., Haag-Wackernagel, D. (2016). Novel mobbing strategies of a fish population against a sessile annelid predator. Scientific Records. accepted.

Prenatal Medicine

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Multimodal regulation of neutrophil NETosis in pregnancy and disturbance in inflammatory conditions

Human pregnancy is associated with a mild pro-inflammatory state, characterized by activation of circulatory neutrophils. We have previously shown that this is disturbed in pathologies such as preeclampsia, leading to excessive neutrophil extracellular trap (NETs) formation. NETs are a rather unique innate immune tool employed by granulocytes, whereby their nuclear DNA is extruded into the extracellular environment to ensnare and kill a wide-array of microorganisms, ranging from bacteria, fungi to parasites. Aberrant NETs formation may damage or induce cell death of surrounding tissues, and is implicated in a number of pathologies including rheumatoid arthritis (RA), SLE, small vessel vasculitis, or coagulopathies. The underlying signal transducing pathway initiating the NETotic process involves calcium mobilization, generation of ROS by NAPDH oxidase, nuclear localization of both neutrophil elastase (NE) and myeloperoxidase (MPO), and citrullination of histones by peptidylarginine deiminase 4 (PAD4). The latter events contribute to chromatin unfolding, a prerequisite for efficient DNA extrusion.

To date no study has examined NETs generation in normal pregnancies, nor which factors could modulate such a response during extensive period of human gestation. For this reason we recently examined NETosis in all three trimesters of normal pregnancy. Our data indicate that neutrophils from normal healthy pregnancies exhibit a distinct pro-NETotic phenotype, which increases towards term. This was characterised by an elevated response to pro-NETotic stimuli, as well as clear elevations in the expression of key signalling components required for efficient NETs formation.

In our studies to ascertain which factors drive this phenotypic change, we determined that G-CSF, the circulatory levels of which also increase in parallel during pregnancy, plays a key role in promoting a progressively enhanced NETotic state. We also noted that early in gestation (1st trimester), NETosis is augmented by the action of human chorionic gonadotropin (hCG), which boosts the action of G-CSF, in promoting a primed pro-NETotic phenotype.

As pregnancy advances, and the levels of hCG subside to be replaced by other steroid hormones expressed by the placenta, namely estrogen (E2) and progesterone (P4), we observed that maternal neutrophil activity is modulated in a considerably more complex manner. In this regard, E2 acts by being pro-NETotic. This action is antagonized by the action of P4, which serves to retain neutrophils in a highly primed state, yet hindering NETs formation. Our data suggest that the regulatory mechanism evoked by P4 involves a blockage of NE localisation to the nucleus, a step previously shown to be vital for efficient NETs formation. Since neutrophil NETs were originally described as an anti-pathogenic mechanism, our data would

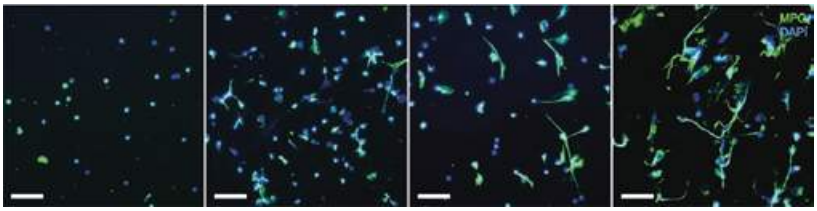

Fig. 1: NET formation and neutrophil pro-NETotic priming are augmented during pregnancy. *In vitro* spontaneous NET formation by neutrophils from healthy pregnant donors over a 3 hour time course by fluorescence microscopy using Immunofluorescence staining for MPO (green) and DNA counterstain with DAPI (blue). Scale bars: 50 µm.

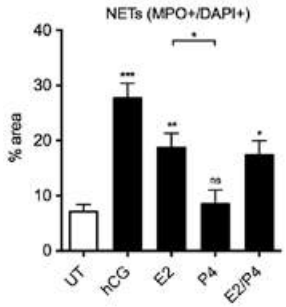

Fig. 2: Neutrophil pro-NETotic priming is regulated by pregnancy hormones.
Morphometric analysis of the NETotic (MPO+/DAPI+) neutrophils from healthy control donors after 2 hours treatment with physiologic concentrations of hCG, E2, P4 and E2/P4.

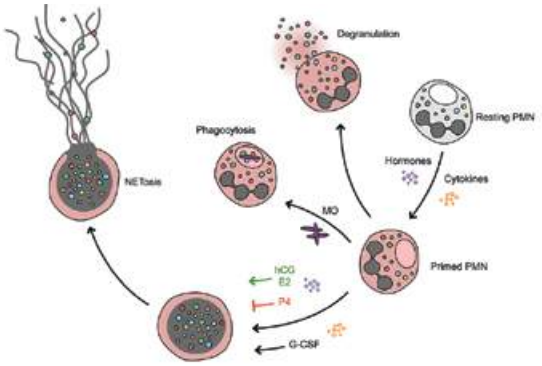

Fig. 3: Progesterone antagonises the estrogen and G-CSF driven neutrophil extracellular trap formation during pregnancy.
During pregnancy neutrophils lie under the increased influence of cytokines, e. g. G-CSF, and sex hormones. This specific milieu appears to poise the neutrophils in a stable pro-NETotic primed state. Depending on the stimulus, for instance microorganisms (MO), neutrophils react by phagocytosis or degranulation. When a different NETotic stimulus is present, such as excessive placentally derived plasma microparticles (MP) in preeclampsia (25), primed neutrophils react with overt NET release. Pro-NETotic combinations of hormones and cytokines are given in green, the most potent in bold green. Inhibitory combinations are given in red, the most potent in bold red.

suggest that this operative arm of the innate immune response is highly pro-active in human pregnancy. In this manner, by being in a highly primed pro-NETotic state, such pre-activated neutrophils could react immediately to a pathogenic threat.
Our data also provide new insight into how aberrancies in this system may contribute to the underlying aetiology of preeclampsia, in that this condition is associated with elevated levels of hCG and G-CSF, which would serve to enhance NETosis. Since there are suggestions that progesterone levels may be reduced in preeclampsia, such an imbalance may trigger an enhanced pro-NETotic response, which is exacerbated by the occurrence of inflammatory placental micro-debris, abundant in this condition.
On the other hand, our data may provide a novel insight into autoimmune conditions such as systemic lupus erythematosus, which is associated with reduced levels of progesterone, both during the menstrual cycle, as well as during pregnancy. Since NETosis is altered in SLE, it is possible that the negative feedback loop hindering NETs formation provided by progesterone contributes to the preeclampsia-like symptoms frequently observed in pregnant women affected by SLE.
These facets are being examined in ongoing studies, together with the Prof. P. Hasler, Aarau

Selected Publications

Gupta A, Giaglis S, Hasler P, Hahn S. (2014) Efficient neutrophil extracellular trap induction requires mobilization of both intracellular and extracellular calcium pools and is modulated by cyclosporine A. Plos One May 12;9(5):e97088

Chowdhury CS, Giaglis S, Walker UA, Buser A, Hahn S, Hasler P. (2014) Enhanced neutrophil extracellular trap generation in rheumatoid arthritis: analysis of underlying signal transduction pathways and potential diagnostic utility. Arthritis Res Ther 16

Wu M, Ries JJ, Proietti E, Vogt D, Hahn S, Hoesli I. (2016) Development of Late-Onset Preeclampsia in Association with Road Densities as a Proxy for Traffic-Related Air Pollution. Fetal Diagn Ther 39:21–27

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Giaglis S, Stoikou M, Sur Chowdhury C, Schäfer G, Grimolizzi F, *et al.* (2016) Multimodal regulation of NET formation in pregnancy: progesterone antagonizes the pro-NETotic effect of estrogen and G-CSF. Frontiers in Immunology 7

Connection to Clinical Practice

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Is NETosis altered in pregnancies complicated by the "Great Obstetrical Syndromes"?
We have previously detected aberrant NETs formation in pregnancies affected by preeclampsia (PE). The key aetological feature driving the development of PE is defect in placentation, specifically a failure in the transformation of maternal spiral arteries from a high pulsatile system to a relaxed low-pressure system. This placental aberrancy in more evident in cases with early onset PE, than those developing PE close to term.
Recent studies have indicated that such placental defects are not restricted only to PE, but are also evident in intra-uterine growth restriction (IUGR), and as suggested by new reports, may even occur in preterm labour (PTL).
Consequently, defects in spiral artery modification may be common aetiological factors in the "great obstetrical syndromes" (GOS) of PE, IUGR and PTL. What is unresolved is how defective placentation contributes to such disparate pathologies.
Our query is to determined whether these defects in placentation evident in the GOS are analogous, or whether only those in early onset PE trigger an overt maternal inflammatory response. As a read-out for our study, we will examine the activity of circulatory maternal neutrophils, particularly their ability to undergo NETosis, as we have observed this to be a reliable marker for inflammation. Hence, our study may shed new light onto similarities or differences between the GOS disorders, and also contribute into the development of new screening markers.
Together with Prof. Gabor Than (Budapest) we are investigating the role of PP13, a placentally derived galectin, in modulating neutrophil activity in PE and other GOS.

Hepatology

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Chronic Hepatitis and Hepatocellular Carcinoma

Worldwide, liver cancer is the sixth most common cancer with an estimated 750'000 new cases annually. 90% of liver cancers are hepatocellular carcinomas (HCCs). Over 95% of HCCs develop on the background of chronic liver disease and indeed, more than 80% of HCCs occur in cirrhotic livers. The most important risk factor for HCC is chronic viral hepatitis caused by infections with hepatitis C virus (HCV) or hepatitis B virus (HBV). Both viruses are parentally transmitted and can cause chronic hepatitis. In the case of HCV infections, the innate immune system in the liver reacts within days after infection with a strong interferon (IFN) driven response. However, despite the upregulation of hundreds of IFN stimulated genes (ISGs), and the recruitment of specific T cells, HCV persists in over 70% of infected individuals. In the chronic phase, ISG expression remains strongly induced in a subset of patients depending on the IFN lambda 4 genotype. A recently discovered genetic polymorphism of the IFN lambda gene locus determines if an individual can or can not produce IFN lambda 4 (reviewed in Heim, M.H., Bochud, P.Y., and George, J. [2016]). Patients with the IFN lambda 4 "deltaG" genotype have persistantly induced ISGs. Somewhat paradoxically, IFN lambda 4 producers are less likely to clear the virus spontaneously, and are less likely to be cured by IFNalpha treatments (reviewed in Heim, M.H. (2013). Combined biochemical, immunological and genetic studies provide compelling evidence that IFN lambda 4 is the central regulator of the innate immune response to HCV (Terczynska-Dyla, E. *et al.* [2014]). In a recent transcriptome analysis we found that the IFN lambda 4 induced ISG induction is qualitatively identical to the response to therapeutically injected pegylated IFN alpha, but significantly weaker. This quantitative difference explains why the endogenous IFN system is ineffective in clearing HCV (Boldanova *et al.* [2017]). The molecular mechanisms that link the IFN lambda 4 genotype with the cellular immune response to HCV are still unclear and presently one of the research focus of the Hepatology Laboratory.

Hepatocellular carcinomas are characterized by very heterogenous clinical courses. It is widely assumed, that genetic and epigenetic heterogeneity underlies the wide variation in clinical presentation, disease progression and response to the different treatments. Within the MERiC research consortium (an ERC synergy grant funded collaboration of 4 research groups in Basel) our laboratory is investigating the molecular diversity of HCCs, and the molecular mechanisms of evasive resistance of HCCs to systemic therapies. (MERiC = mechanisms of evasive resistance in cancer). We realised that transcriptome based molecular classifications systems of HCCs have severe limitations and are clinically not useful (Makowska *et al.* [2016]). Ongoing efforts for a molecular classification therefore will integrate genomic, transcriptomic and proteomic data obtained from tumor biopsies (Dazert *et al.* [2016]).

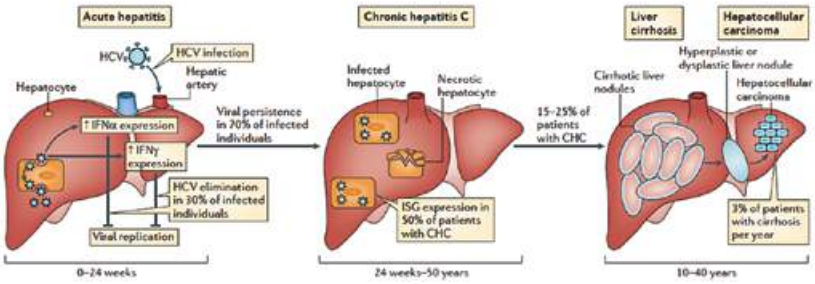

Figure 1: Natural history of CHC. Hepatitis C virus (HCV) enters the liver through the hepatic artery and the portal vein, which are the two blood vessels that transport blood into the liver. Acute HCV infection lasts from 0 to 24 weeks and often remains undetected. Approximately 70% of HCV-infected individuals develop chronic hepatitis C (CHC). Most patients do not develop substantial liver fibrosis or clinically relevant liver disease. However, in 15–25% of the cases, cirrhosis develops over 10–40 years. Decompensated cirrhosis and hepatocellular carcinoma are the most important causes of mortality in end-stage CHC. IFN, interferon; ISG, IFN-stimulated gene.

Selected Publications

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Immuno-biology

Basic and translational aspects of lymphocyte function and its metabolic basis

T cells belong to the adaptive arm of the immune system and play key roles in protecting the host from invading pathogens. The metabolic repertoire of T cells – which encompasses metabolic enzymes/pathways, the available nutrient sensors and metabolic checkpoint kinases, and the epigenetic programming of metabolic genes – directly enables and modulates specific immune functions (Gubser/Bantug *et al.*; Dimeloe *et al.*; Jones and Thompson; MacIver *et al.*; O'Sullivan *et al.*; van der Windt *et al.*).

The signaling pathways that are shaping the metabolic repertoire of T cells *in vivo* remain poorly defined, particularly in humans. We established a critical link between the complement system and immunometabolic adaptations driving CD4+ T cell effector function. In activated human T cells, autocrine stimulation of the complement receptor CD46 was found to be required for enhanced expression of the glucose transporter GLUT1 and induction of the amino acid transporter LAT1. Furthermore, CD46 activation simultaneously drives expression of LAMTOR5, which mediates assembly of the amino acid-sensing Ragulator-Rag-mTORC1 complex, and increases glycolysis and oxidative phosphorylation required for cytokine production (Kolev/Dimeloe *et al.*).

The cell-intrinsic metabolic repertoire is also subject to modification by extracellular local and systemic metabolic alterations – driven e. g. by malignancies or infection, respectively. In that regard, depletion of glucose from the tumor-microenvironment by malignant cells has been shown to impair effector-functions of tumor-infiltrating T cells (Zhao, E. *et al.*, 2016). Systemic metabolic alterations, such as increased abundance of the short-chain fatty acid acetate during bacterial infections (i.e. acetate stress-levels), likewise impact immune cell metabolism and function. Specifically, we found that acetate is taken up by memory CD8+ T cells, metabolized and utilized to acetylate GAPDH, which in turn increases glycolytic flux and thereby the memory T cell recall capacity (Balmer *et al.*, 2016).

Our ongoing goal is to delineate the molecular basis of how cellular metabolism is regulated, and itself regulates, immune-function in health and disease states, and to define how environmental cues are integrated at the cellular level by immune cells to shape cellular metabolism and function.

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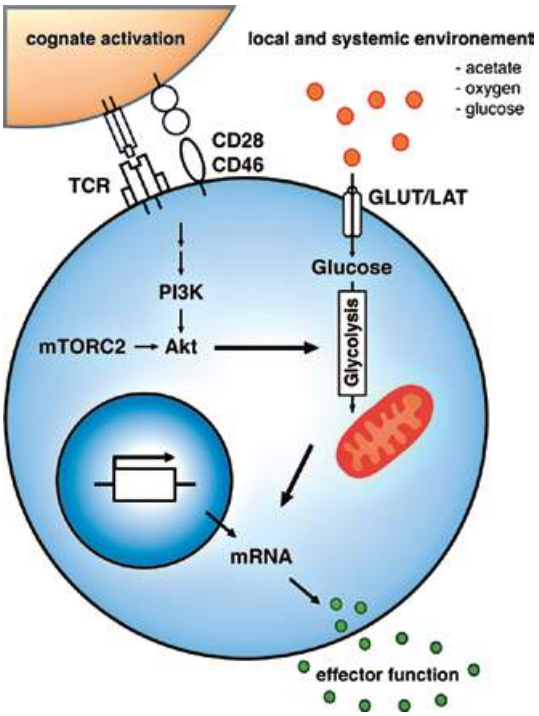

Fig. 1: T cell metabolism defines cellular function – and is, itself, influenced by the extracellular metabolic environment. Glucose metabolism and mitochondrial function are central to the immune function of T cells, critically regulated through PI3K–Akt–mTOR signaling. Activation of T cells initiates rapid metabolic reprogramming (increased glycolysis and oxidative phosphorylation) and changes the expression of key nutrient channels, such as glucose and amino acid transporters. TCR=T cell receptor; CD28 and CD46 (a complement receptor) represent co-stimulatory molecules; GLUT=glucose transporter; LAT=amino acid transporter

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Transplantation and Clinical Virology

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Translational Research in Clinical Virology: From Bedside to Bench and Back to the Patients

"Transplantation and Clinical Virology" is interested in translational research of virus infections to improve clinical diagnosis, prevention, and treatment. This includes

- Respiratory viruses (RV);
- Human herpesviruses; and
- Human polyomaviruses in vulnerable populations (e.g. HIV/AIDS; transplantation; autoimmunity; inherited immunodeficiency).

We aim at characterizing 1) key determinants of virus pathology; 2) potential targets of antiviral intervention; 3) adaptive immune responses; 4) modifiable and non-modifiable risk factors in patients.

The focus on human polyomaviruses (HPyVs) serves as example: In the last decade, 12 new HPyVs have been identified by molecular methods in addition to BKPyV and JCPyV known since 1971. Currently, at least 5 HPyVs have been convincingly linked to diseases, all in immuncompromized patients. Importantly, no specific antivirals are available for the treatment of HPyV disease making immune reconstitution the main stay of any therapeutic approach today.

The polyomavirus non-coding control region (NCCR) harbors the origin of viral DNA genome replication and promoter/enhancers controlling the sequential bi-directional expression of PyV early and late gene expression. We reported that kidney transplant patients with persistent BKPyV viremia showed the emergence of viral variants with rearranged NCCR. We demonstrated that the emerging rr-NCCR caused an activated early viral gene expression, higher viral loads, and replication rates (replication capacity) *in vitro*, and more advanced disease in patients. A similar dynamic change of the NCCR was seen in JCPyV of HIV patients with PML linking activated early viral gene expression to replication and pathology. Importantly, non-rearranged JCPyV NCCR is activated by HIV1 explaining the high number of PML among HIV/AIDS patients.

We conducted extensive point mutation analyses of the archetype BKPyV NCCR identifying 3 phenotypic groups whereby Sp1 affinity and orientation governed bi-directional BKPyV early and late gene expression (Fig. 1). The pathologic relevance of the point mutations was supported by their identification in clinical isolates from patients with nephropathy and hemorrhagic cystitis. Thus, similar to HIV/tat in JCPyV, BKPyV (re-)activation does not only result from failure of immune control, but also from activation from the NCCR.

Based on clinical studies, BKPyV viremia and nephropathy has been associated with tacrolimus as main calcineurin inhibitor. We observed that cyclosporine A inhibited/slowed BKPyV replication, whereas tacrolimus activated/accelerated BKPyV replication *in vitro*. Importantly, viral activation by tacrolimus was antagonized by sirolimus competing the intracellular binding protein FKBP-12 (Fig. 2). The data strengthen the theme that activation of virus replication plus failure of adaptive immune control synergize in the emergence of opportunist viral infections in immunocompromised hosts. This knowledge could be taken back to the clinical management of BKPyV not only by reducing immunosuppression, but by switching to low-dose cyclosporine plus mTOR inhibitor combinations.

Characterizing BKPyV T-cell responses through IGRAs to 15mers, we observed that BKPyV Vp1 responses were generally stronger and involved mostly CD4 T-cells, whereas LTag responses were weaker, but contained a larger fraction of specific CD8 T-cells in peripheral blood. Since CD8 T-cells are the main cytotoxic effectors, and since LTag are rate limiting for BKPyV replication *in vitro* and *in vivo* (see NCCR mutant variants), we hypothesized that LTag-specific CD8 T-cells are key effectors of antiviral immunity. We identified 39 of 90 predicted immunodom-

inant 9mers responses that will be developed for T-cell vaccines (Fig. 3), and for clinical assays to guide immosuppression reduction.

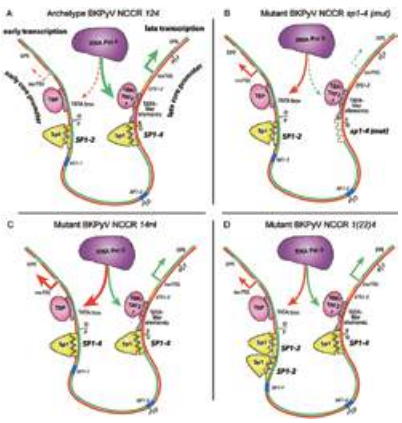

Fig. 1: The host cell factors Sp1, TATA- and downstream core promoter elements form imperfect symmetry underlying bidirectional expression of the early and late viral gene region of BKPyV (see Bethge *et al.* 2016 J. Virol 90: 10083–10101).

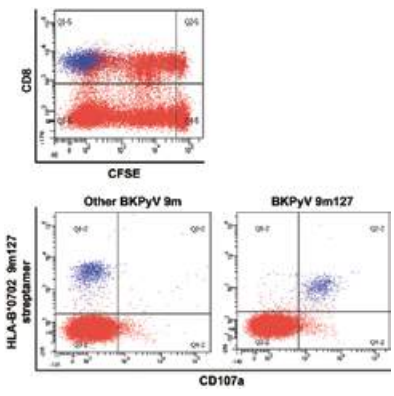

Fig. 3: Characterization BKPyV-specific CD8 T-cell responses identifying immune-dominant 9mers epitopes (see Cioni *et al.* 2016 Am J Transplant 16: 1193–1206). CD8+ T cell proliferation (CSFE dye) and cytotoxic degranulation (CD107a) of HLA-9mer-streptamer CD8 T-cells (blue).

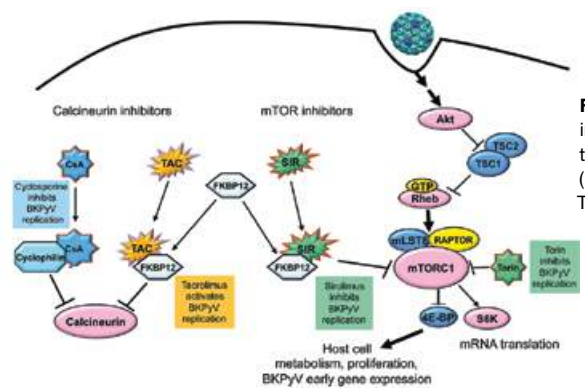

Fig. 2: Effects of calcineurin inhibitors and mTOR inhibitors on BKPyV replication (see Hirsch *et al.* 2016 Am J Transplant 16: 821–832).

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Ljungman P, Boeckh M, Hirsch HH, Josephson F, Lundgren J, Nichols G, Pikis A, Razonable RR, Miller V, Griffiths PD. Definitions of Cytomegalovirus Infection and Disease in Transplant Patients for Use in Clinical Trials. Clin Infect Dis. 64(1):87–91

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Connection to Clinical Practice

Nina Khanna, Manuel Battegay, Joerg Halter, Ulrich Heininger, Jakob Passweg, Stefan Schaub, Jürg Steiger, Daiana Stolz, Michael Tamm

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Multidisciplinary Clinical Virology Approach to Improve Outcome in Vulnerable Patients

Together with Prof Nina Khanna and Prof M. Battegay; and Profs J Passweg, and J. Halter, a retrospective study on respiratory viruses (RV) in stem cell transplant recipients was conducted with special focus on the therapeutic role of oral ribavirin. Also, we are participating in a multi-center randomized phase 3 study of a fusion inhibitor targeting respiratory syncytial virus in HCT recipients with upper or lower respiratory tract infections (GS-US-218-0108; GS-US-218-1502).

Together with Profs D. Stolz and M. Tamm, a prospective study of RV-multiplex testing in patients exacerbations of asthma and COPD. In a 5-year prospective study with Prof J Gavalda, Spain, we defined the role of RV for acute and chronic lung disease after lung transplantation.

Together with PD MJ Kim, Prof S. Schaub and the Swiss Transplant Cohort Study (STCS), we are engaged in a prospective randomized trial (Dr O. Manuel, Lausanne; https://clinicaltrials.gov/ct2/show/NCT02538172) to study the role of CMV-specific T-cells in guiding the duration of valganciclovir prophylaxis. In collaboration with colleagues from Finland, USA, Italy, Spain, and USA, we aim at improving the traceability and commutability of CMV load tests to define the path forward to uniform criteria for FDA and EMA-approved clinical CMV studies for licensing antivirals and vaccines.

Together with the STCS, we organised a multicentre study to identify the BKPyV-specific T-cells in kidney transplantation. By the end of the study, more than 1800 EliSpot assays from PBMCs will inform about super-dominant epitopes and their MHC-I context to improve risk assessment and immunosuppression reduction.

Pediatric Immunology

The Immunobiology Of The Thymus

T cell responses play a crucial role in providing protective immunity. At the same time the effector molecules and cells of this defense system can also be responsible for a broad range of autoimmune pathologies when directed against an individual's own tissues. Lineage commitment and maturation of T cells is instructed during the cell's intrathymic development and result from a physical and functional interaction with the stromal microenvironment. Thymic epithelial cells (TEC) constitute an essential component of this stroma whereby cortical (c) and medullary (m) TEC have distinct structural, antigenic and functional features. cTEC provide signals that commit hematopoietic precursor cells to a T cell fate and select those immature T cells for further differentiation that express a functionally competent and for the individual largely useful T cell receptor (TCR). In contrast, mTEC contribute to the establishment of self tolerance via the expression of peripheral tissue-specific antigens (PTA).

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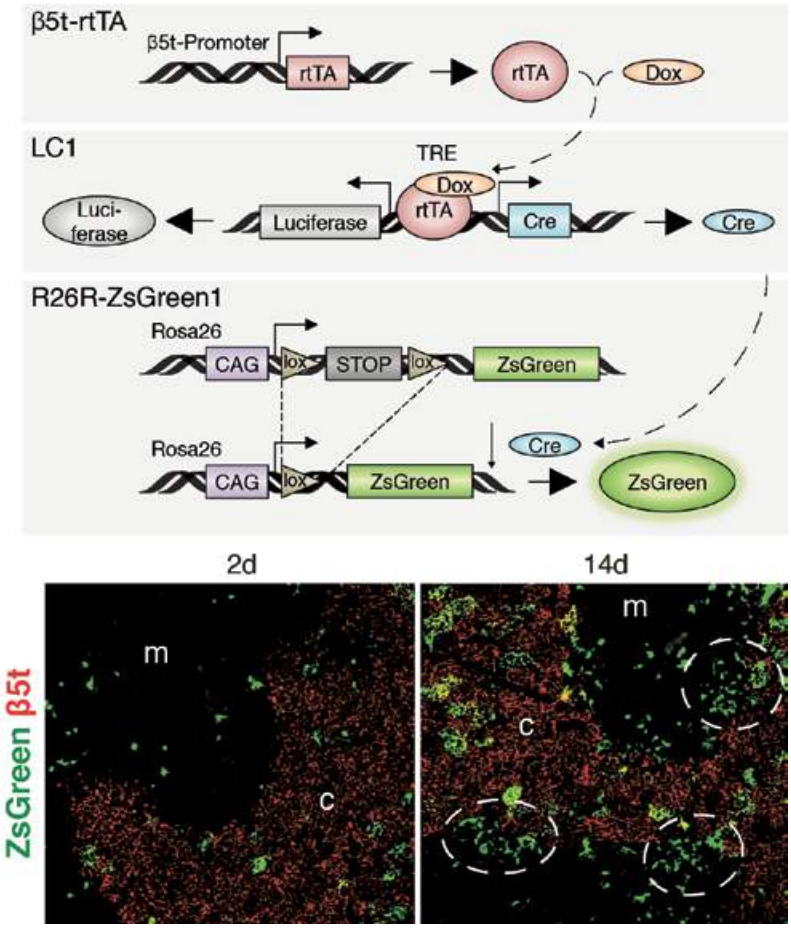

Fig. 1: Upper panel: Schematic representation of triple transgenic inducible reporter mice (3xtg^{β5t}). Lower panel: Immunohistologal analysis of the thymus from 3xtg^{β5t} mice that had been treated with doxycycline at 1-week of age and followed for 2 and 14 days, respectively. Cryostat sections were analysed for the expression of β5t (red) and ZsGreen (green). Note that the majority of ZsGreen positive cells are positioned at the cortical-medullary junction. c: cortex, m: medulla

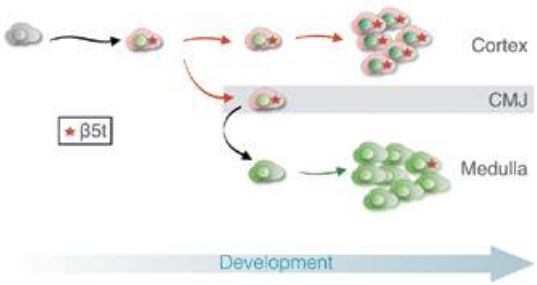

Fig. 2: Single β5t⁺ epithelial progenitor cells located at the cortico-medullary junction (CMJ) adopt a medullary TEC fate and actively contribute post-natal medulla growth.

The research of the laboratory of Paediatric Immunology focuses on a detailed understanding of: (i) the genetic and epigenetic control of TEC development and function. We have generated specific genetic gain and loss of function mouse models that allow a precise interrogation of particular mechanisms relevant for thymus organogenesis and function. Our research has defined the direct target genes of the TEC master regulator transcription factor Foxn1 as well as it's DNA binding motif. We detailed the function of FOXN1 and demonstrated that in addition to the transcriptional control of genes involved in attraction and lineage commitment of T cell precursors, FOXN1 also regulates genes involved in antigen processing and thymocyte selection. Thus, critical events in thymic lymphostromal crosstalk and T cell selection are choreographed by FOXN1. Further studies have characterized the importance of the polycomb repressive complex 2 (PRC2) for regular TEC biology. DNA methylation and the generation of micro-RNA constitute additional epigenetic mechanisms that we have identified to play an essential role in TEC fate, maintenance and function, including the expression of PTA. The expression of some of these PTA is controlled by the nuclear protein Autoimmune Regulator (AIRE). A single cell transcriptome analysis of TEC subsets proficient or deficient in the expression of histone and DNA modifying enzymes further revealed that PTA expression by thymic epithelia is dependent on different epigenetic mechanisms.
(ii) the identity of TEC stem/precursor cells and their intermediate stages in differentiation towards mature cTEC and mTEC. Using *in vivo* lineage fate mapping, we demonstrated that β5t+ cTEC-like progenitors give rise to the medullary TEC compartment. Lineage-tracing demonstrated that the postnatal medulla is expanded from individual β5t+ cortical progenitors located at the cortico-medullary junction. These results therefore not only define a developmental window during which the expansion of medulla is enabled by progenitors resident in the cortex, but also reveal the spatio-temporal dynamics that control medulla growth.
(iii) the role of metabolic pathways in TEC biology. We have generated mice lacking in TEC essential components of the mTOR complex. Absence of mTORC1 signalling ensued a decrease in mTEC numbers that correlated with reductions in mitochondrial mass, respiration, endoplasmic reticulum network and reactive oxygen species. Furthermore, it lowered the affinity of positively selected TCRs and led to reduced negative selection. Hence, our findings link bioenergetic changes in TEC to specific stages in T cell development.
(iv) the consequences of altered thymus development on T cell function, in particular regulatory T cells (Treg). Thymic hypoplasia and ensuing peripheral lymphopenia favor the expansion of a particular potent Treg subset that can be identified by CD103 and ICOS. This phenotype represents a lymph node specific differentiation stage that requires the availability of antigen. Thus, we showed that tissue-resident cues determine the overall potency of the peripheral Treg pool by shaping its subset composition.

Selected Publications

Zuklys S, Handel A, Zhanybekova S, Govani F, Keller M, Maio S, Mayer CE, Teh HY, Hafen K, Gallone G, *et al.* (2016) Foxn1 regulates in postnatal thymic epithelial cells key target genes essential for T cell development. Nat. Immunol. In press

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Molecular Immune Regulation

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Towards a better molecular understanding of T cells as a basis for new therapeutic concepts

The mammalian immune system is important to fight infections but it also fulfills many more functions, some of which are perhaps underappreciated. For instance, immune cells are involved in tolerance to the growing fetus, wound healing and the removal of old and altered cells such as tumors. To achieve these diverse goals the immune system is composed of many cell types and soluble molecules. Due to its complexity and power of effector mechanisms many layers of regulation ensure proper control over what is attacked when and where. Any mistakes can lead to severe clinical disease.

Modern medicine relies in many ways on the immune system. Vaccines induce protective immune responses and cell and organ transplantation between genetically different individuals is made possible through immunosuppressive therapies. At times therapeutic immune regulation seeks to exploit the very same cellular and molecular pathways to achieve opposing goals. As an example, certain drugs exploit a molecule called “CTLA-4” to dampen the immune response (used in organ transplantation) while different drugs targeting the same molecule can enhance immune responses to fight cancer (“checkpoint inhibitors” in cancer immunotherapy).

Therefore, we are interested in a better understanding of how molecular mechanisms control such intricate regulation. We are particularly interested in an immune cell type called “T cells”. We investigate how different T cell subsets are controlled genetically. In the past few years we have focused on a class of small RNAs called microRNAs (miRNA) which regulate other genes posttranscriptionally. We found that miRNAs are dynamically controlled in response to many signals including signals required for T cell activation (costimulation). Furthermore, many studies revealed that one miRNA can control many genes but often each gene is only mildly regulated. We have focused our work on a miRNA locus called miR-17-92. We demonstrated that miR-17-92 is important for regulatory T cells (Treg) which dampen immune responses and follicular helper T cells (T_{FH}) which help B cells generate better antibodies. Although we contributed to a better molecular understanding why miR-17-92 is required for T_{FH} differentiation and function many potentially regulated gene candidates remain to be investigated.

In order to study the genetic networks which are controlled by individual miRNAs more comprehensibly we needed to develop novel tools. To this end our group recently developed a new CRISPR/Cas-based genome engineering protocol for efficient gene editing in T cells. This new approach enables us to introduce very small, defined genetic changes in the genome of T cells. We are now planning to use this new approach to accelerate our miRNA studies. In parallel we have started a collaboration with Dr. Mihaela Zavolan from the Biocentre to generate molecular maps of 3’ untranslated regions (3’UTRs). miRNAs most frequently interact with the 3’UTRs of their target genes and the lengths of 3’UTRs can vary. Therefore we will use the newly generated maps as a basis to design perturbation experiments using the gene editing protocol.

In collaboration with Dr. Christoph Hess we are also investigating how miRNAs control cell intrinsic metabolism. Moreover, we are searching for small molecules which could be used to modulate miRNA function. Such molecules would be useful research tools and could potentially be used for therapeutic goals.

Finally, based on our initial results obtained with genome engineering tools we are convinced that this technique will open completely new fields which were unimaginable only a few years ago. We envision using this novel genetic toolbox for studies in human cells including investigations of patient-specific mutations. Therefore we are actively pursuing efforts into this uncharted territory.

Connection to Clinical Practice

Prof. Jürg Steiger
Nephrology and Transplantation Immunology

Our lab is associated with the Transplantation Immunology & Nephrology clinics of the University Hospital Basel. We will collaborate with Prof. Jürg Steiger to translate our basic research interests in clinical settings. In addition we are establishing collaborations with other clinicians to use the power of genome engineering in human samples.

Selected Publications

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Infection Biology

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Host pathogen interaction in infectious diseases

The infection biology research group explores host- and pathogen-specific aspects of infectious diseases in a strong translational setting. Our main focus is to understand the interaction of innate and adaptive immune responses in the context of bacterial, fungal and viral infections with the aim at elucidating key mechanisms of immune protection, thus fostering personalized treatment – with the overarching goal to improve clinical outcome.

Host response to fungal infections

Invasive fungal infections are devastating in immune compromised patients and associated with high mortality rates despite treatment. Risk assessment and diagnosis remain a major challenge leading to administration of broad-spectrum antifungal prophylaxis and over-treatment, which in turn is associated with high costs, drug interactions and, most importantly, limited clinical efficacy with emergence of resistant strains. Hence, reliable tools to identify patients at risk and tailored treatment strategies to improve patient outcome are urgently needed.

We recently found that functional neutrophil, NK-cell and *Aspergillus fumigatus*-specific Th1 immunity is associated with a better outcome in patients after allogeneic hematopoietic stem cell transplantation (HSCT) with invasive aspergillosis (Fig. 1A).

To get a broader knowledge about immunological factors increasing the susceptibility to fungal infections in other patient populations, we further studied risk factors for development of *Candida* esophagitis in HIV-infected patients. We found that HIV-1-infected patients with *Candida* esophagitis had an accumulation of multiple, partly *Candida*-specific immunological defects (Fig. 1B). In addition, long-term immune recovery was impaired even after introduction of combination antiretroviral therapy, illustrating that specific immunological gaps persist in these patients. These data clearly support the rationale for early combination antiretroviral therapy initiation to prevent irreversible immune defects.

Adoptive T-cell therapy for infectious diseases

Adoptive transfer of pathogen-specific donor-derived T cells is to date the most promising and feasible immunotherapeutic strategy in transplant recipients restoring the lacking T-cell function. Its potential as prophylactic or therapeutic treatment has been demonstrated for viral infections after transplantation. We recently initiated a clinical trial of adoptive T-cell therapy for treatment-refractory viral infections with cytomegalovirus, Epstein Barr virus and adenovirus after HSCT using the cytokine capture assay® (Miltenyi Biotec) at the University Hospital of Basel (ClinTrials ID NCTO2007356), which is so far unique in Switzerland.

The fact that higher *Aspergillus fumigatus*-specific Th1 responses correlate with a better clinical outcome in HSCT recipients with invasive aspergillosis encourages the development of antifungal T-cell transfer. We recently identified immunogenic *Aspergillus fumigatus* antigens, which are likely protective by inducing Th1-cell responses in healthy individuals and HSCT recipients with well-controlled invasive aspergillosis (Fig. 2A,B). We were further able to select these antigen-specific cells based on activation-dependent expression of CD137 or CD154 with a GMP-compliant protocol (Fig. 2C). Moreover, cell lines containing T cells specific for all three proteins cross-reacted to the most relevant human-pathogenic molds (Fig. 2D). These results greatly foster adoptive T-cell therapy for these problematic infections.

Staphylococcal implant infections

Bacterial infection of implanted devices is a major health care problem occurring in about 5 % of patients. These infections are mainly caused by biofilm-forming staphylococci, which are generally tolerant to antibiotic treatment. We could recently demonstrate that silver-coating can efficiently prevent implant-associated infections and thus can be considered for clinical application (Fig. 3). Further studies are on the way investigating the host pathogen interaction in biofilm-associated infections.

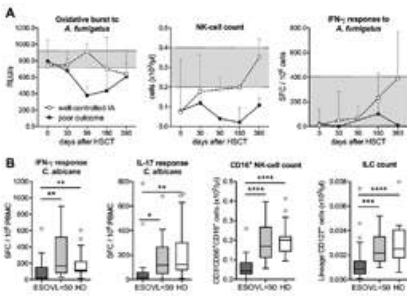

Fig. 1: Antifungal immune response in patients with invasive aspergillosis after allogeneic hematopoietic stem cell transplantation (A) and HIV-1-infected patients with *Candida* esophagitis (B). RLU/s = relative light units per second, IA = invasive aspergillosis, ILC = innate lymphoid cell count, ESO = *Candida* esophagitis, VL < 50 = viral load < 50 c/ml HD = healthy donors For details: Stuehler C, J Infect Dis. 2015 Sep 15;212(6): 959–67; Stuehler C and Bernardini C et al, AIDS May 5, 2016.

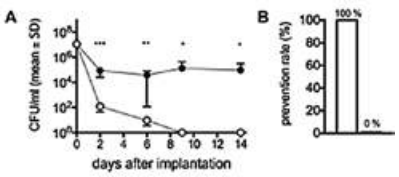

Fig. 3: Infection of uncoated (closed circles) or silver-coated (open circles) TiAlNb tissue cages with *S. epidermidis* strain 1457 in C57BL/6 mice. Planktonic bacteria indicated as colony forming units (CFU) per cage in the tissue cage fluid after perioperative infection (A). Prevention rates for silver-coated (open columns) or uncoated (closed columns) tissue cages (B). TiAlNb = titanium-aluminum-niobium alloy (Kuehl R, Antimicrob Agents Chemother 2016 Mar 25;60(4):2467–75).

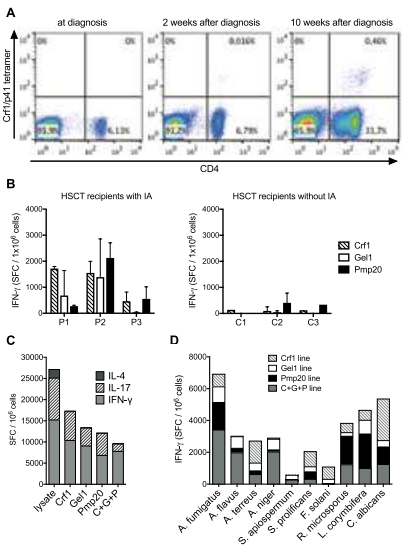

Fig. 2: Hematopoietic stem cell transplant (HSCT) recipients with active invasive aspergillosis (IA) show expansion of Crf1/p41-specific cells over time (A) and respond to *Aspergillus fumigatus* antigens irrespective of absolute CD4+ T-cell counts (B). The antigen-specific T-cell lines can be expanded (C) and show cross-reactivity to different filamentous molds (D). IFN-γ = interferon-gamma, PBMC = peripheral blood mononuclear cells, C + G + P = all antigens Crf1, Gel1 and Pmp20 were used for stimulation (J Infect Dis. 2015 Apr 15;211(8):1251–61).

Connection to Clinical Practice

Fungal and viral infections have become a leading cause of morbidity and mortality in immunosuppressed patients. Pharmaceutical agents are often less effective in the setting of immunodeficiency, may cause substantial side effects, are expensive and may generate resistance. To overcome these issues, understanding the host-pathogen interaction and exploring strategies such as adoptive T-cell transfer that boost and induce long-term immunity may be promising in these patients.

Selected Publications

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Immune Cell Biology

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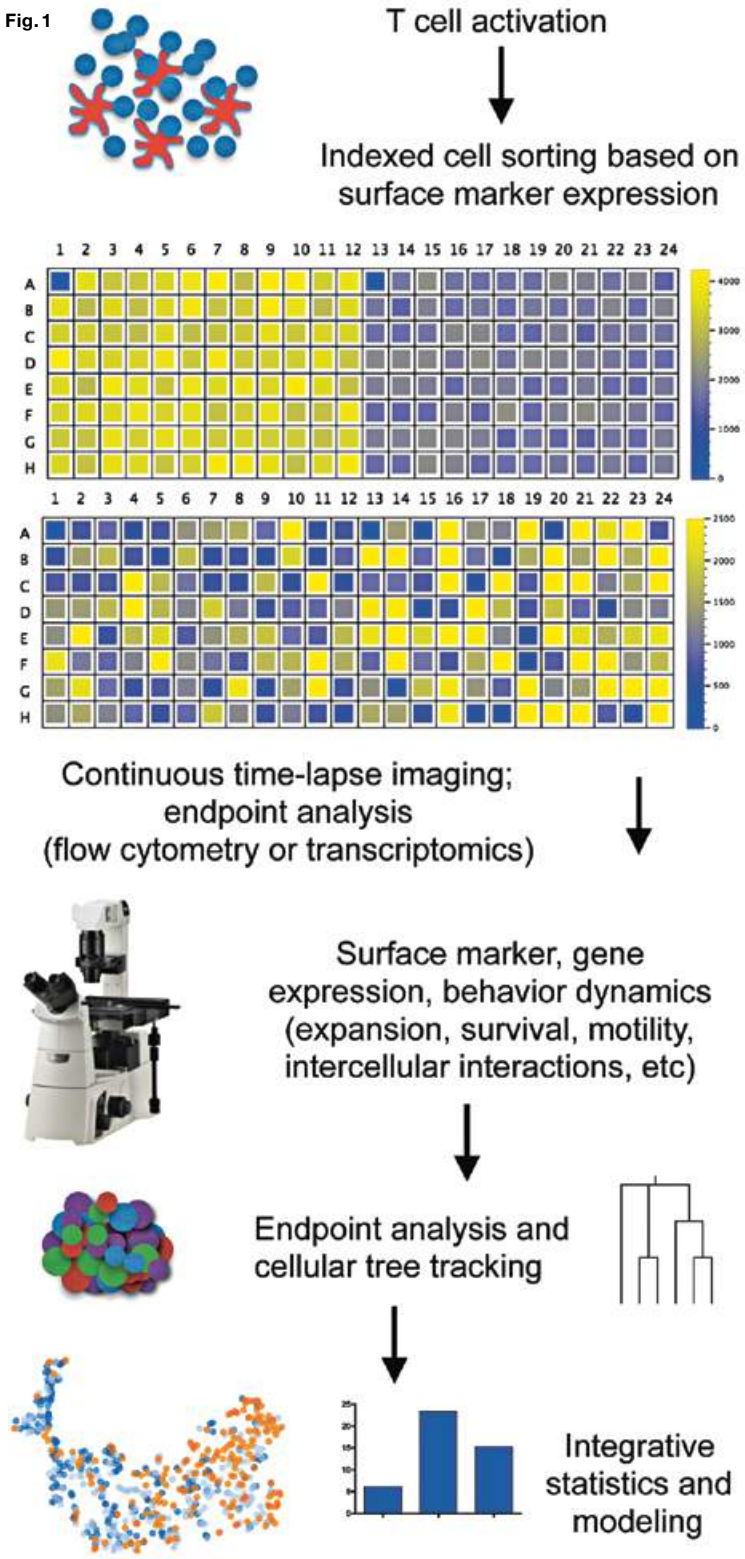
Fig. 1



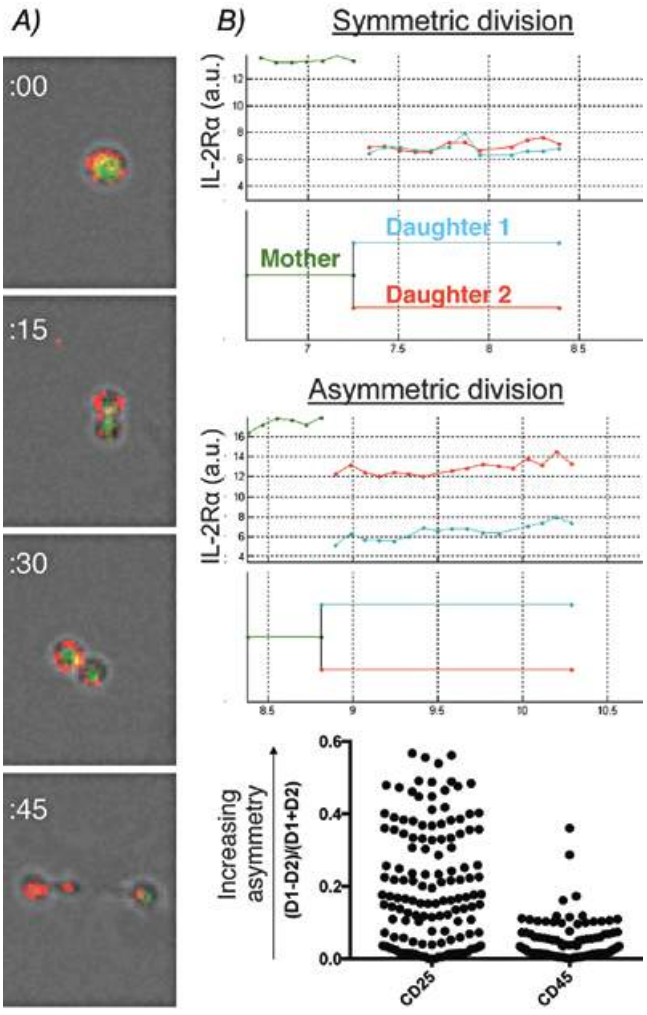


Fig. 2:

A) Dynamic T cell interactions can be tracked from the onset of activation and beyond the first division. In this time series IL-2Rα (red) is asymmetrically partitioned into daughter cells during mitosis while GFP is symmetrically partitioned. Time is noted in minutes. **B)** Quantification of IL-2Rα on paired daughter cells shows that IL-2Rα can be unequally transmitted to daughter cells during division. Simultaneous quantification of surface CD45 demonstrates symmetric distribution of this receptor concurrent with asymmetric IL-2R.

Regulation and dynamics of T cell fate

Our immune systems have evolved to provide host protection against a wide range of infections. CD4+ T cells are unique in their ability to differentiate into a variety of pathogen appropriate and specialized effector and memory subsets. A long-standing question in T cell biology is how this diversity is achieved. The goal of our research is to understand: how do individual T cells give rise to progeny with such diverse cell fates?

Individual T cells are inherently variable. On the other hand, T cell differentiation is a dynamic process and requires the integration of multiple signaling inputs including antigen, cytokines and nutrients, over time. Several models have been developed to explain T cell diversification but most have relied on either population level analyses or static, snapshot measurements. Although highly informative, these approaches cannot fully capture the complexity of T cell behavior or shed light on the specific mechanisms underlying flexible and specific T cell responses. To address these issues, we use continuous time-lapse microscopy to monitor the temporal expression of surface markers and transcription factors expressed by single T cells cultured under distinct differentiation conditions. By reconstructing the phenotypic and proliferative history of divergent T cell family trees, we aim to generate new predictive models that describe the process of T cell diversification.

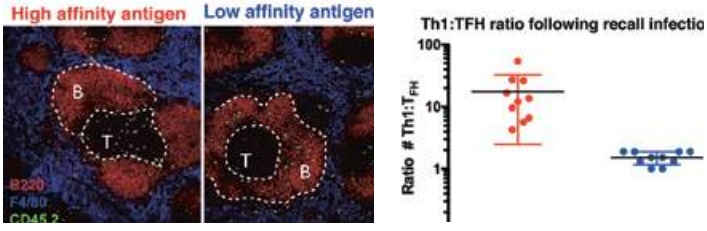
These efforts are complemented by *in vivo* models to address T cell differentiation during acute bacterial infection, chronic viral infection and tolerance. We are particularly interested in understanding how dynamic access to antigen and cytokines regulates the flexibility and plasticity of effector and memory CD4+ T cells. Here we are focused on two broad questions: (i) how does signaling through the T cell receptor regulate the quality and extent of CD4 differentiation; and (ii) what are the molecular mechanisms that link T cell receptor signal strength with the generation of heterogeneous CD4+T cell fates? By linking complex T cell phenotype, behavior and fate across time, our goal is to define and uncover novel molecular pathways underlying T cell fate decisions. We believe that our work to address fundamental biologic questions will lay a foundation for innovative solutions to a wide range of public health challenges.

Selected Publications

Keck S, Schmaler M, Ganter S, Wyss L, Obserle S, Huseby ES, Zehn D, King CG. (2014) Antigen affinity and antigen dose exert distinct influences on CD4 T-cell differentiation. Proc. Natl. Acad. 111(41):14852–7

Fig. 3:

T cell differentiation during infection. **A)** High affinity activated T cells primarily differentiate into "inflammatory" Th1 cells that home to T cell areas; low affinity activated T cells strongly differentiate into follicular helper T cells (TFH) that provide help to B cells making antibodies. **B)** Memory T cells generated by high affinity activation maintain a Th1 bias while memory T cells generated by low affinity activation maintain a TFH bias. In both cases, memory T cells are recalled with high affinity antigen. These data suggest that effector T cell lineage can be programmed by the strength of primary stimulation and that memory T cells have reduced Th1 cells TFH cells flexibility.



Molecular Virology

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Immunologic HIV control during continuous, highly effective therapy promotes tropism-dependent loss of infected cells

In the past decades successful therapy has completely changed the global landscape of HIV infection: New drugs with excellent side-effect profiles are today available for long therapy and a perspective of life-long virus suppression; now the role of regained or retained immune functions is back in the focus of interest. Can the improved immune capacity reduce virus spread and disease? Yet, despite all therapeutic developments the unavoidable flip-side of decade-long therapy continues to be the increased risk of drug-resistant HIV and treatment failure. While most of the responsible mutations in the respective therapy target genes have been characterized, cases with persisting "low level viremia" or virologic failure without overt mutations remain puzzling. Therefore, a better understanding of alternative resistance mechanisms, e.g. via mutations in enzyme substrates may prove clinically important. As practical, clinical contributions of our research-driven molecular work, and as elements of capacity building towards improved clinical care my research group got engaged in projects with rural settings of our globe. Our efforts intend to address aspects of the ambitious WHO 90-90-90 aim towards ending the global HIV epidemic: Identify by year 2020 globally 90 % of all HIV infected people, provide treatment to 90% of them with virus suppression in 90 %. The current focus of ongoing work in my research group is thus on three key questions:

1) How can immune function complement effective HIV therapy?
We have recently discovered that recovering immune functions can crucially change the fate of HIV-1 in a given patient. Such competence might assist therapeutic strategies to arm the natural defense over time in concert with HIV drug therapy. Published work revealed that HIV tends to change its host tropism during the course of infection: Early, close to 90% of patients harbor mostly HIV that uses the chemokine receptor CCR5 for cell entry, late in the disease up to 50 % of viruses are CXCR4-tropic. CXCR4-use therefore seemed to indicate the emergence of more aggressive virus variants, characteristic for late-stage disease. We set out to probe this hypothesis by a longitudinal follow-up of patients in the Swiss HIV cohort study by deep-sequencing approaches. To our surprise, and against expectation the study demonstrated the opposite trend: In patients with long term suppression of virus replication, CCR5-tropic HIV variants re-appeared or remained, while CXCR4 tropic virus in most cases drastically declined (Fig. 1), obviously as result of therapeutic pressure. This new finding correlates well with other key properties of CXCR4-viruses: lower envelope glycosylation, stronger induction of inflammation and cell activation. It is thus likely that immune-stimulating features enable preferable control and elimination of CXCR4-tropic HIV variants and –infected cells under therapy.

2) Does HIV resistance evolve under long-term therapy?
Although combination therapy has become superbly successful for the global fight against HIV, escape from therapy continues to be a serious issue for all inhibitor classes, including the highly potent protease inhibitors (PI). Mechanistically most PI-resistances correlate with genetic changes within the protease gene, i.e. drug-specific mutations or those that confer broad viral resistance to most drugs of the class. However, clinical situations are observed, where no such obvious link can be established. Our investigations identified genetic changes in the gag gene for structural HIV proteins, which serve as protease substrate. Fig. 2a details H-bridges between enzyme and substrate that are crucial for proper interaction and Gag-

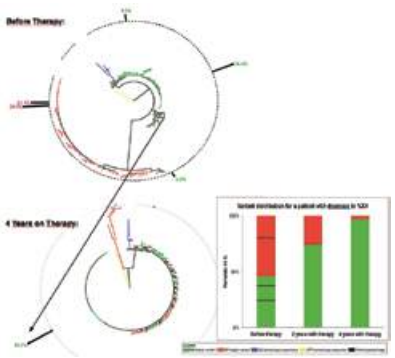

Fig. 1: Next generation sequencing-based phylogenetic tree before and during treatment for a representative patient. The population of CXCR4-tropic variants declines massively. Green dots represent CCR5-tropic HIV-variants in the virus population, red dots CXCR4-tropic ones. Blue: position of V3 consensus sequences of subtypes A, B, and C. In yellow: consensus HIV-1 subtype B LTR sequence used for rooting. The most prominent outgrowing variant under therapy is marked with a black arrow. Black bars indicate the frequency (in percent) of a given variant in the entire HIV sequence pool. The bar graph summarizes the relative frequencies of the 5 most prominent viruses over time.

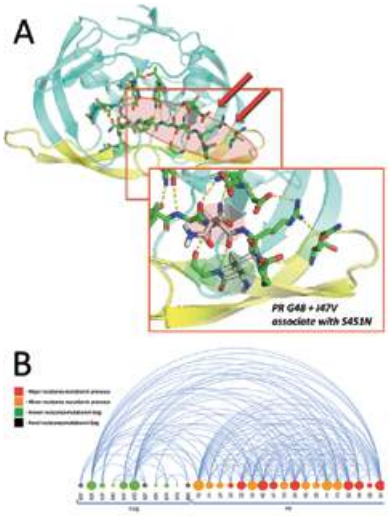

Fig. 2A: HIV-1 Protease complexed with the decapeptide at the p1/p6 Gag substrate junction **(top)**. Specific polar contacts of Gag S451 with PR G48 mediates the immediate neighboring of mutated position I47V with Gag S451N under therapy **(bottom),** which causes a simultaneous loss of a polar contact between PR D30 and PR Q58 (next to PR flap region). Altered residues are in purple. Polar contacts between substrate and enzyme are shown as yellow dots; arrows indicate position of lost polar contacts. **Fig. 2B:** Arch-diagram depicting the interactions between residues in Gag and Protease (PR). Blue lines as well as sizes of dots indicate the number of connections of a given amino acid (numbers indicate position) with other residues. The most active positions correlate with known or suspected drug resistances.

processing. We analyzed data in the resistance database of the Swiss HIV Cohort Study for identifying more of the possible links. As summarized in the arch diagram in Fig. 2b several links between protease and Gag were unraveled that associate with therapy. Currently their role is being verified by phenotyping in cell-based assays in the presence of inhibitors. This may help identify new clinically relevant mutations to be considered in HIV resistance testing

3) Modern diagnostic tools and patient care in rural Africa.

With a translational, practical effort my research group is involved in the establishment and implementation of quantitative viral load-testing and the genotypic determination of HIV drug resistance in a rural, resource-limited setting. In collaboration with N. Labhardt and the Swiss NGO SolidarMed we study therapy efficacy, viral resistance-emergence as well as the successful retention in antiviral care over time in the North of Lesotho. Up to here the project was already able to demonstrate that nurse-led hospitals can achieve, similar to Swiss settings, about 90% treatment success. Pleasantly surprising, also patients' therapy adherence is similarly high (Fig. 3). However, in very sharp contrast to the positive adult situation, virus control and retention in care for children and adolescents were shown to pose massive challenges. Therefore, along with our most recent introduction of sensitive virus-load measurement and sequence-based resistance testing in Butha-Buthe/Seboche we are currently concentrating our work on new concepts to accompany and improve clinical work towards long-term virus suppression for these key populations and the overall health towards prolonging the lives of the most-affected in a country that, with an overall prevalence of 20% HIV-1-positivity, is among those hardest hit by HIV/AIDS.

Connection to Clinical Practice

Niklaus Labhardt
STPHI, Basel

Molecular tools for HIV diagnostics in order to reach the WHO goals of "90-90-90" in rural Africa
(See point 3 in the abstract to the left)

Selected Publications

Bader J, Däumer M, Schöni-Affolter F, Böni J, Gorgievski-Hrisoho M, Martinetti G, Thielen A, Klimkait T, and the Swiss HIV Cohort Study. Therapeutic Immune Recovery and Reduction of CXCR4- Tropic HIV-1. Clinical Infectious Diseases 10.1093/cid/ciw737, Advance Access published Nov. 12, 2016

Bader J, Schöni-Affolter F, Böni J, Gorgievski-Hrisoho M, Martinetti G, Battegay M, Klimkait T & the Swiss HIV Cohort Study. (2016) Correlating HIV tropism with immunological response under combination antiretroviral therapy. HIV Med. 2016 Mar 17. doi: 10.1111/hiv.12365

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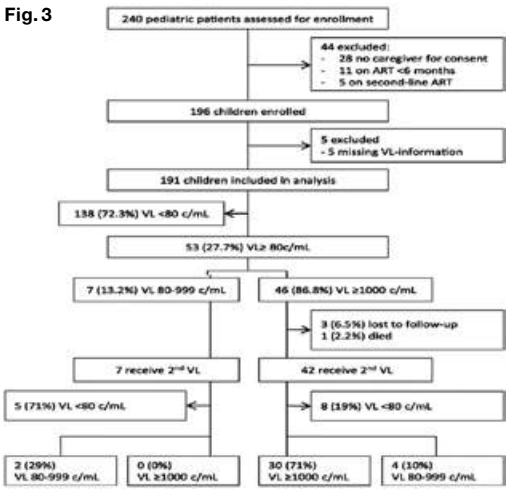
Fig. 3



Experimental Rheumatology

The regulation of innate immune mechanisms by microRNA in the development of chronic arthritis

The laboratory of experimental rheumatology has been newly established in January 2015 at the Petersplatz location of DBM. Our general research interest is the role of the innate immune system in the pathogenesis of inflammatory rheumatic diseases, including chronic arthritis. Activation of receptors of the innate immune system, such as Toll-like receptors (TLR), has been shown to occur at an early stage of synovitis in patients with rheumatoid arthritis (RA)(Ospelt *et al.*, 2008). Increasing evidence indicates that inflammatory pathways are controlled by post-transcriptional mechanisms . Epigenetic control of gene expression by microRNA (miR) was demonstrated to be dysregulated in RA (Duroux-Richard *et al.*, 2011; Stanczyk *et al.*, 2008). In our work we have focused on miR regulation of TLR-dependent inflammatory pathways in synovial fibroblasts and monocytes/macrophages. *In vitro* TLR-ligand stimulated synovial cells and monocyte-derived macrophages showed differential expression of miRNA in low-density array analysis. Candidate miRs were confirmed by real-time PCR and possible target genes in the TLR-signaling pathway predicted in silico. A set of miR were identified that were found to be regulated by TLR-ligands. Expression of miR and their target genes are measured in blood or synovial fluid of patients with rheumatoid arthritis. To assess the functional effects of the candidate miRs, they are overexpressed in primary human synovial cells or monocyte-derived macrophages from the peripheral blood. Alternatively, miR antagonists are used to inhibit miR function in these cells. Subsequently, production of proinflammatory cytokines and chemokines as well as proliferation and apoptosis are measured.
We aim at a better understanding of the contribution of dysregulation of miR expression to the pathogenesis of chronic joint inflammation.

Duroux-Richard, I., Presumey, J., Courties, G., Gay, S., Gordeladze, J., Jorgensen, C., Kyburz, D., and Apparailly, F. (2011). MicroRNAs as new player in rheumatoid arthritis. Joint Bone Spine *78,* 17–22.
Ospelt, C., Brentano, F., Rengel, Y., Stanczyk, J., Kolling, C., Tak, P.P., Gay, R.E., Gay, S., and Kyburz, D. (2008). Overexpression of toll-like receptors 3 and 4 in synovial tissue from patients with early rheumatoid arthritis: toll-like receptor expression in early and longstanding arthritis. Arthritis Rheum *58,* 3684–3692.
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Selected Publications

Hruska-Plochan M, Li B, Kyburz D, Krutzfeld J, Landmesser U, Aguzzi A, Polymenidou M. (2015) New and emerging roles of small RNAs in neurodegeneration, muscle, cardiovascular and in ammatory diseases. Swiss Med Wkly 145, w14192
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Kyburz D, Finckh A. (2013) The importance of early treatment for the prognosis of rheumatoid arthritis. Swiss Med Wkly 143, w13865
Niederer F, Trenkmann M, Ospelt C, Karouzakis E, Neidhart M, Stanczyk J, Kolling C, Gay RE, Detmar M, Gay S, *et al.* (2012) Down-regulation of microRNA-34a* in rheumatoid arthritis synovial fibroblasts promotes apoptosis resistance. Arthritis Rheum 64, 1771–1779

Connection to Clinical Practice

Biomarker analysis for outcome prediction in early arthritis patients

We are conducting studies in patients with early stage rheumatoid arthritis to identify biomarkers that are related to disease progression and response to therapy. Biomarkers include cytokines, disease-associated autoantibodies and microRNA. Together with clinical and imaging characteristics we aim at improving prediction of disease outcome.

Translational Neuro-immunology

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Single cell lymphocyte migration in multiple sclerosis

Individual cell-migration is fundamental to protective immunity but plays also a central role in the pathogenesis of autoimmune diseases. Our general aim is to assess migration characteristics of primary human T cells within specific subpopulations on a single cell level in health and in autoimmunity.

Orchestrated migration of T cells provides the basis for their precise positioning within lymphoid and non-lymphoid tissues. Conversely, migration of autoreactive lymphocytes is fundamental to the pathogenesis of autoimmune diseases, such as multiple sclerosis (MS). The importance of T cell migration in the pathogenesis of MS is supported by the fact that two highly efficacious drugs for the treatment of MS (fingolimod and natalizumab) act on T cell migration.

Most of our insight into T cell migration is based on the monitoring of bulk cell populations in animal models. For human T cells, a relatively limited set of migration-characteristics have been recapitulated *in vitro*, again mostly by using bulk cell populations and probing the peripheral blood lymphocyte compartment. While important understanding of mechanisms underlying T cell migration derives from these studies, a potential heterogeneity of migration between individual cells is not interrogated using this approach. Also, clinical samples containing only small numbers of T cells are not amenable for analysis using classical *in vitro* migration assays. Specifically, understanding the migration characteristics of T cells derived from cerebrospinal fluid of MS patients –the closest routinely accessible approximation to pathogenic cells in this disease– would be of particular interest.

In recent years, introduction of microfabrication technologies resulted in the generation of so-called microfluidic devices, which allow monitoring of cells on a single-cell level with high spatio-temporal resolution. We have developed microfluidic devices specifically designed to studying the migration characteristics of primary human T cells. These devices allow a highly precise build-up of soluble and immobilized chemokine gradients. Characterizing migration properties of individual human CD4+ memory T cells *ex vivo*, we have already established the proof of concept that this technique can overcome previous limitations of standard migration assays. With the help of microfluidics we aim at assessing migration characteristics of immune cells derived from the cerebrospinal fluid of patients with MS and relate these findings with clinical phenotypes.

Selected Publications

Schwarz J, Bierbaum V, Merrin J, Frank T, Hauschild R, Bollenbach T, Tay S, Sixt M, Mehling M. A microfluidic device for measuring cell migration towards substrate-bound and soluble chemokine gradients. Sci Rep. 2016 Nov 7;6:36440

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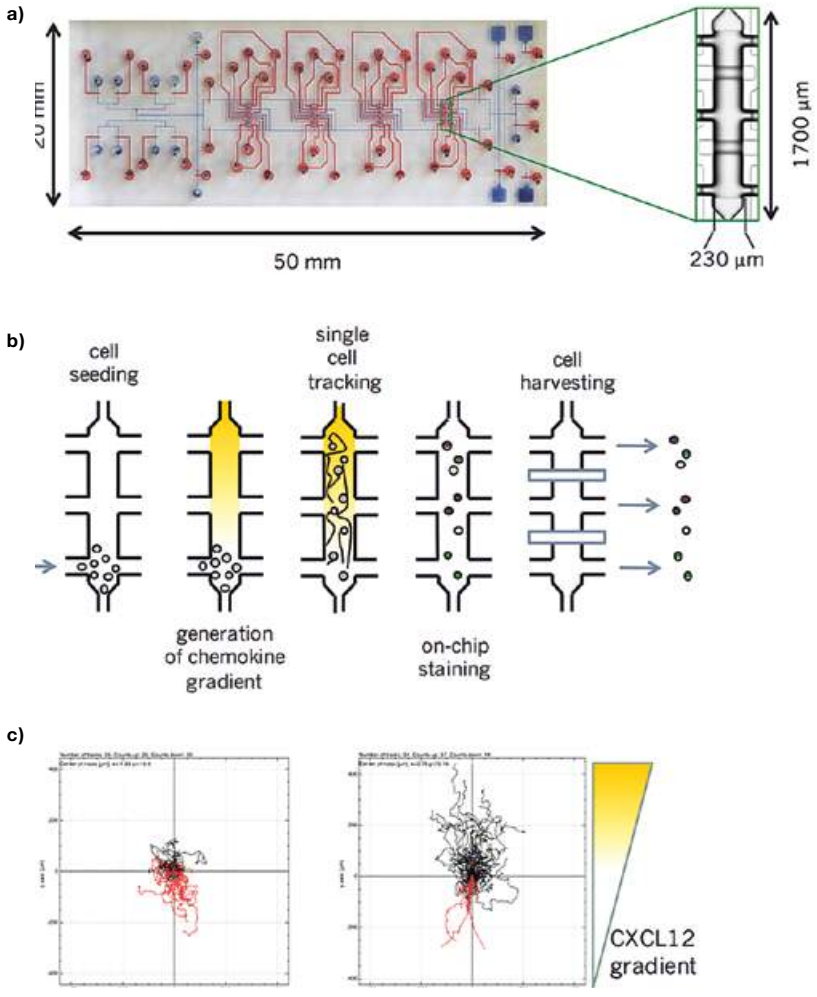

Fig. 1: Overview and geometry of microfluidic chemotaxis and cell retrieval device. **(a)** Actual photograph of the device (blue structures: flow channels; red structures: control channels) and one magnified migration chamber. **(b)** Schematic overview of the functionality of an individual microfluidic migration chamber for assessing single cell migration in highly controllable chemokine gradients. **(c)** Trajectories of migrating primary human CD4+ central memory T cells plotted on a common starting point in the absence (left panel) and presence (right panel) of a CXCL12 gradient.

Connection to Clinical Practice

Prof. Dr. Ludwig Kappos
Prof. Tobias Derfuss
Neurology Department

Relating migration-characteristics of MS cerebrospinal fluid T cells with clinical phenotypes

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS). The disease affects worldwide over 2.5 million individuals and is the most common cause of neurological disability in young adults. Autoreactive immune responses in the CNS, mediated by encephalitogenic lymphocytes are thought to play central roles in the initiation and propagation of MS pathology. Priming of encephalitogenic T cells presumably occurs in secondary lymphoid tissues (SLT) outside the CNS. Thus, migration of these cells – or their precursors – to SLT, and from SLT to the CNS are crucial steps in the immunopathogenesis of MS. To assess migration characteristics of T cells derived from patients with MS our Translational Neuroimmunology Laboratory works in close collaboration to the Division of Neurology of the University Hospital Basel, which includes a MS Outpatient Clinic. This provides access to samples from a unique and well defined population of MS-patients with different stages and courses of the disease and different treatments including experimental therapies in clinical development.

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Mononuclear phagocytes in mucosal immune responses

Inflammatory bowel diseases (IBD), such as Crohn`s disease and ulcerative colitis, are chronic relapsing diseases with increasing incidence and prevalence in the developed world. Innate and adaptive immune responses to constituents of the intestinal microbiota are essential for the development of IBD. Intestinal mononuclear phagocytes are in direct contact with the intestinal microbiota, initiate innate immune responses and shape adaptive immune responses. Phagocytes are located in the lamina propria of the gastrointestinal tract, in cryptopatches and isolated lymph follicles in close proximity to innate lymphoid cells (ILCs). Infection of animals with the mouse pathogen C. rodentium leads to IL-22 production by ILCs, which regulates the expression of REG family proteins required for the defense to infections with enteric pathogens. The depletion of phagocytes decreased IL-22 production by ILCs. This means that phagocytes support IL-22 production by ILCs required for host defense in the gut (Manta et al, Mucosal Immunol, 2013).

Mononuclear phagocytes may also initiate adaptive immune responses in the gut as indicated by the close proximity of phagocytes to T cells in the lamina propria of the gastrointestinal tract. Colonic phagocytes sample continuously fluorescent labelled E. coli. To reduce the complex interactions between phagocytes and T cells in presence of the intestinal microflora with vast array pf potential antigens, an antigen-specific colitis model was developed. In this model the challenge of E. coli expressing the antigen ovalbumin induces colitis in immunodeficient animals (RAG animals) reconstituted with antigen-specific T cells. *Ex vivo* confocal imaging allows the visualization of phagocytes that have sampled E.coli in proximity to T cells. *In vitro* studies indicated that the phagocytes deliver the antigen to dendritic cells, which migrate to mesenteric lymph nodes. In mesenteric lymph nodes the dendritic cells prime T cells, which home back to the lamina propria. In the lamina propria phagocytes are able to activate the effector T cells (Rossini et al, Mucosal Immunol, 2014).

These findings support the hypothesis that mononuclear phagocytes in the gastro-intestinal tract are of importance for the sampling of constituents of the microbiota, the initiation of innate and adaptive immune responses. Likely, mononuclear phagocytes play a key role in the pathogenesis of IBD.

Selected Publications

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Schulz O, Ugur M, Friedrichsen M, Radulovic K, Niess JH, Jalkanen S, Krueger A, Pabst O. (2014) Hypertrophy of infected Peyer's patches arises from global, interferon-receptor, and CD69-independent shutdown of lymphocyte egress. Mucosal Immunol. 7, 892–904

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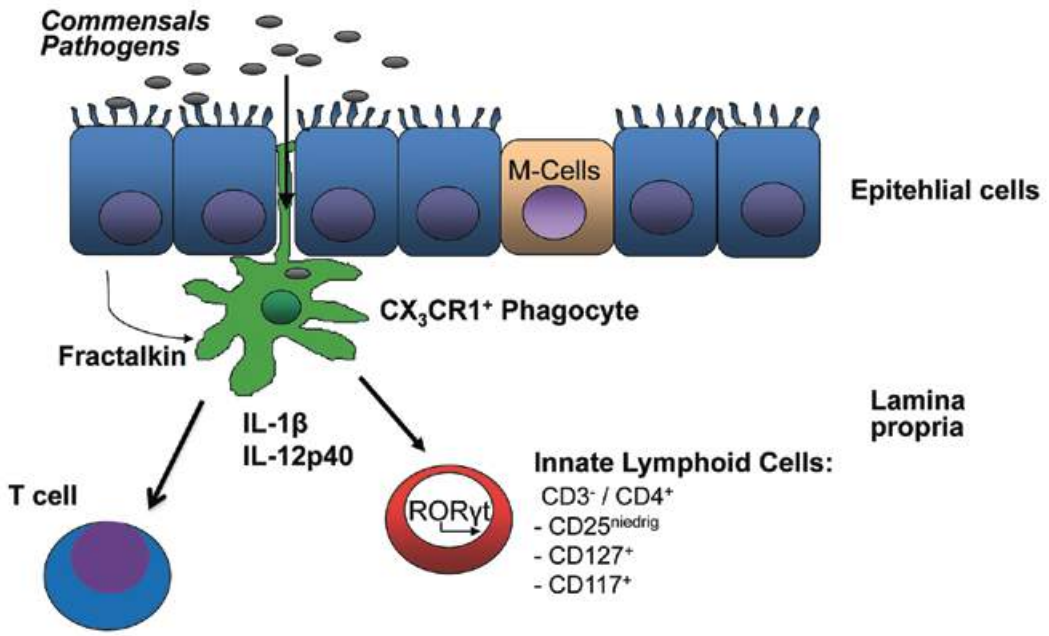

Fig. 1: Mononuclear phagocytes sample constituents of the intestinal microflora, initiate effector T cell responses (adaptive immunity) and facilitate IL-22 production by innate lymphoid cells (innate immunity).

Transplantation Immunology and Nephrology

Retired during report period

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Dr. Caroline Wehmeier (Postdoc)
Dr. Lena Wyss (Postdoc)

Understanding the principles of naturally occuring T cell tolerance

One of the central mysteries of immunology is self-tolerance. How does the human body select ~10e12 T lymphocytes, that are reactive to foreign pathogens but tolerant to normal cellular constituents of the host? The work of our laboratory seeks to understand the general principles by which a healthy individual's immune system achieves a state of self-tolerance. We are particularly interested in how a tolerant T cell repertoire is selected during development and how it's maintained during adult life. The knowledge derived from our research may eventually impact organ transplantation and autoimmune diseases.

Over the last few years, we demonstrated that the affinity threshold for negative selection is a constant for all thymocytes expressing MHC I restricted TCRs. This binding affinity threshold (KD = 6 μM; estimated T1/2 ≈ 2 sec) is a fundamental biophysical parameter used by developing CD8 lineage cells to establish a tolerant T cell repertoire. More recent experiments indicate that thymocytes destined to enter the CD4 lineage use an affinity threshold (KD = 300 μM; estimated T1/2 ≈ 0.1 sec) for negative selection that is 10-50 fold lower than that used for their CD8 lineage counterparts. These differences are explained by the fraction of the corresponding co-receptor (CD8 or CD4) which carries the initiating kinase, lck. 1% of CD8 vs 10% of CD4 molecules are loaded with lck. The emerging picture is that MHC I restricted thymocytes require a high affinity (longer duration) interaction between the TCR and the self-antigen to initiate negative selection because so few (< 1%) of the CD8 co-receptor molecules carry lck. MHC II restricted thymocytes on the other hand undergo negative selection with a much lower affinity (shorter duration) interaction because a higher proportion (10%) of the CD4 molecules carry lck. Imaging studies and molecular modeling have provided evidence that an antigen engaged TCR must undergo hundreds of collisions with the relevant co-receptors to eventually engage a co-receptor molecule which actually carries the initiating kinase, lck. Based on the number of molecules and the biophysical parameters describing their movement, we developed a mathematical model which describes a mechanism where the TCR can actually "read" antigen affinity and establish an affinity (antigen dwell time) threshold for self-tolerance.

We also examined the affinity threshold required for the induction of experimental autoimmune diabetes. This involves the activation of the integrin LFA-1, formation of long-lasting T cell - antigen presenting cell conjugates, asymmetric cell division and differentiation into short-lived effector cells. Related to this, we are examining the origin of autoimmune T cells; they frequently express threshold affinity TCRs, which are inefficiently removed by clonal deletion. We are also trying to define the minimum number of T cells required to initiate an autoimmune disease.

Another focus of the laboratory is to understand the basic biology of regulatory T cells. Our experiments support the idea that Helios+ FoxP3+ regulatory T cells are survivors of negative selection in the thymus. This implies that the TCR repertoire expressed on Helios+ regulatory T cells is high affinity anti-self. *In vitro* experiments demonstrated that Tregs require contact with MHC II expressing APCs and IL-2 from conventional T cells to proliferate. We are also using monoclonal Tregs and monoclonal Tconv cells to examine the basis of Treg mediated suppression. An additional project focuses on the role of regulatory T cells in maintaining peripheral tolerance.

Finally, we studied the role if MHC II expression in intestinal epithelial cells. In mice specifically lacking MHC II expression in this cell type, we observed an epithelial lymphocytosis.

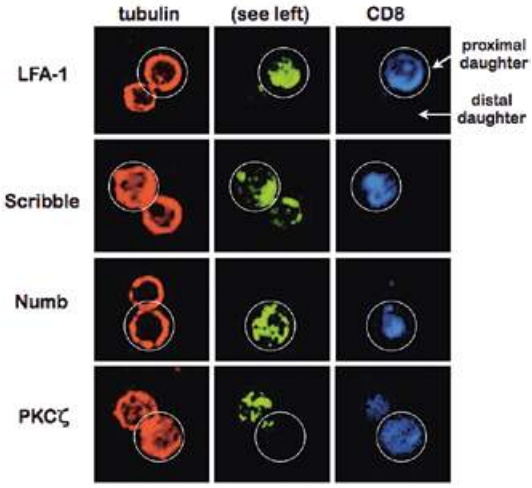

Fig. 1:
Asymmetric T cell division following stimulation with high affinity antigen.

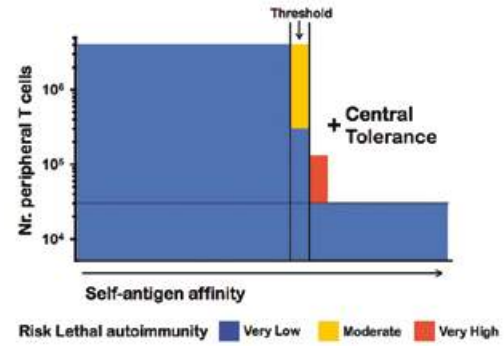

Fig. 2:
Relationship between self-antigen affinity, number of antigen specific T cells and risk of developing experimentally induced autoimmune diabetes.

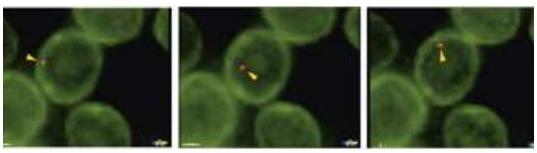

Fig. 3:
Dwell times of single pMHC antigen molecules labeled with quantum dots determined using confocal microscopy.

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Mallaun M, Zenke G, Palmer E. (2010) A discrete affinity-driven elevation of ZAP-70 kinase activity initiates negative selection. Journal of Receptors and Signal Transduction. 30(6), 430–43.

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Connection to Clinical Practice

Prof. Dr. med. Jürg Steiger
Clinic of Transplantation and Immunology

Advances in nephrology research

Jürg Steiger heads the Clinic for Transplantation Immunology and Nephrology and leads a team of 7 clinical nephrologists and 6 fellows, which oversees 60 new kidney transplantations, follow-up of 600 transplanted patients, 16'000 dialyses as well as 1300 in- and out-patient consultations each year. The team covers basic, clinical and translational research in different areas.*

Min-Jeong Kim's research interest is IgA-nephropathy; she explores signaling in mesangial cells and the clinical impact of differentially glycosylated IgA. Andreas Jehle and his team investigate molecular mechanisms of podocyte function in health and disease. Michael Dickenmann introduced ABO-incompatible living donor kidney transplantation to Basel and investigates clinico-pathological outcomes in these patients. Patrizia Amico, Patricia Hirt-Minkowski, Gideon Hönger and Stefan Schaub study the clinical significance of donor-specific HLA-antibodies and explore novel biomarkers for non-invasive monitoring of renal allograft recipients. Jürg Steiger heads the Swiss Transplant Cohort Study (STCS), a multicenter cohort study of all solid organ recipients in Switzerland. The STCS integrates all information on transplant activities providing a basis for high quality clinical research. The data center of the STCS is led by Michael Koller. Finally, Christa Nolte, Felix Burkhalter and Jürg Steiger analyze short and long-term outcomes of living kidney donors in the Swiss organ living donor health registry (SOL-DHR).

* www.unispital-basel.ch/das-universitaetsspital/bereiche/medizin/kliniken-institute-abteilungen/transplantationsimmunologie-nephrologie/lehre-forschung/forschung/).

Experimental Virology

Immunity and pathogenesis in viral infection

Our research interests are centered around the interplay between virus and host, with special emphasis on persistent infection. In broad terms we investigate the following aspects thereof:

- B cell responses in persistent viral infection
- Role of alarmins in T cell immunity
- Virally vectored vaccines
- Viral triggers of autoimmune disease
- Mechanisms of viral pathogenesis

Thereby, our research portfolio covers both adaptive and innate immune defense, with viral infection as a common theme. We combine molecular virological techniques ("reverse genetics") for the engineering of infectious viruses with state-of-the-art mouse infection models, cutting-edge cellular immunological techniques and a broad range of molecular analytics. Although fundamental by character, the questions addressed have strong links to major unmet global health needs. In the mid- to long- term, this offers translational potential, notably for vaccination and treatment of persistent viral diseases such as human immunodeficiency virus (HIV), hepatitis B and C virus, as well as for select autoimmune disorders and cancer.

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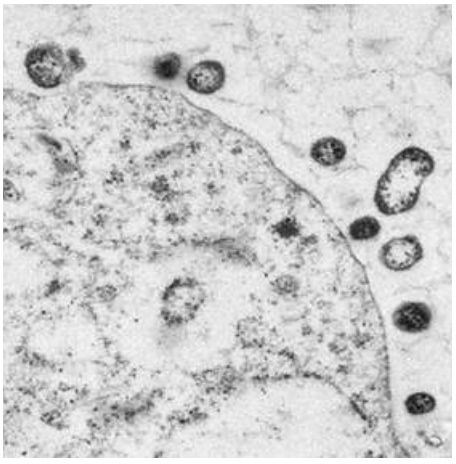

Fig. 1: Electron micrograph of lymphocytic choriomeningitis virus particles budding from a host cell.

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Selected Publications

Fallet B, Narr K, Ertuna YI, Remy M, Sommerstein R, Cornille K, Kreutzfeldt M, Page N, Zimmer G, Geier F, Straub T, Pircher H, Larimore K, Greenberg PD, Merkler D, Pinschewer DD. Interferon-driven deletion of antiviral B cells at the onset of chronic infection. Science Immunology, 2016 October 21;1 (4)

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Immuno-deficiency

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Primary Immunodeficiency

Primary immunodeficiencies (PID) are a rapidly evolving group of genetically determined diseases of the immune system associated with susceptibility to infection and/or autoimmunity. To date, more than 300 different PID have been characterized.

Deficiency of the recombination activating genes (RAG) is associated with PID affecting number and function of both T and B lymphocytes. RAG-PID associated clinical phenotypes range from pediatric severe combined immunodeficiency (SCID) to adult-onset RAG-associated granulomas and autoimmune disease. While complete loss-of-function mutations lead to RAG-dependent SCID, so-called hypomorphic RAG mutations are associated with the late-onset RAG-associated diseases.

The mechanisms involved in autoimmunity and immune-dysregulation due to hypomorphic RAG-mutations are poorly defined. Autoimmunity and immune-dysregulation are also associated with RAG-independent PID, implying that PID and autoimmunity are fundamentally linked.

One main focus of the lab is to analyze in murine models how gradual RAG dysfunction impacts on immunity to infectious pathogens and at the same time to the formation of autoimmunity. This may help identifying mechanisms involved in the generation of autoimmunity in general.

Connection to Clinical Practice

Mike Recher, Christoph Hess
Immunodeficiency Clinic, Medical Outpatient Unit, University Hospital Basel

Molecular mechanisms in and personalized treatment of patients with primary immunodeficiency

Patients with suspected immunodeficiency or immune dysregulations are clinically evaluated in the Immunodeficiency Clinic of the Medical Outpatient Unit of the Basel University Hospital. If needed, patients are treated with supplementation of immunoglobulins and if available treatment specific immunologic treatment.

Since 2015, patients with the diagnosis of primary (genetically determined) immunodeficiency are included into a prospective cohort. Following informed consent, a standardized documentation of the physical status of the patient is combined with analysis of a standardized set of immunological lab data. In addition, the immuno-metabolism of sorted T and B lymphocytes is assessed in a collaboration with the Immunobiology Lab of Christoph Hess. Whole exome sequencing is performed and analyzed in a collaboration with Alexander Navarini, Dermatology, University Hospital Zurich. This allows us to prospectively study the disease course but also to determine the molecular mechanism of disease and to treat the patients in a personalized manner taking into account the molecular mechanism of disease.

Currently, more than 70 patients have been included into the prospective cohort. Identified specific PID-entities include CTLA-4 deficiency, BAFFR mutations and SP110 deficiency.

Selected Publications

Navarini AA, Hruz P, Berger CT, Hou TZ, Schwab C, Gabrysch A, Higgins R, Frede N, Padberg Sgier BC, Kämpe O, Burgener AV, Marquardsen F, Baldin F, Bigler M, Kistner A, Jauch A, Bignucolo O, Meyer B, Meienberg F, Mehling M, Jeker LT, Heijnen I, Daikeler TD, Gebbers JO, Grimbacher B, Sansom DM, Jeker R, Hess C and Recher M. Vedolizumab as a successful treatment of CTLA-4 associated autoimmune enterocolitis. Journal of Allergy and Clinical Immunology, 2016, in press

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Developmental and Molecular Immunology

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Molecular mechanisms guiding hematopoietic cell development

A permissive role for IL-7 in mouse B cell development

Patients deficient for IL-7 or the IL-7R are practically devoid of T cells but have normal B cell compartments. In mice deficient for IL-7 or the IL-7R both T cell and B cell compartments are dramatically reduced. Based on these findings it was argued that IL-7 plays a redundant role in human B cell development but a non-redundant and may be even an instructive role in mouse B cell development. Now we challenged this hypothesis by analyzing the by us generated FLT3L transgenic mice. These transgenic mice posses dramatically increased populations of progenitor cells in the bone marrow including the pre-pro-B cells. Now by generating IL-7 deficient mice on a FLT3L transgenic background we found a very significant rescue of all the precursor B cell subpopulations in the bone marrow and the mature B cell populations in the periphery. Thus, increased levels of FLT3L make the role of IL-7 in mouse B cell development largely redundant. Our findings rather suggest that IL-7 mainly acts as a growth factor for pre-B cells and thereby making B cell development more efficient. Yet, another suggestion coming from our studies is that human B cell development is regulated by FLT3L.

Hematopoietic progenitors with multipotent developmental potential: real multipotency or heterogeneity?

Over the past couple of years new hematopoietic progenitors with multipotent developmental potential mainly determined *in vitro* were described. However, by the generation of so-called reporter mice the multipotency of some of these precursors *in vivo* was questioned. Moreover, the multipotent developmental potential *in vitro* of these precursors was tested at the “bulk” but not at the single cell level. Therefore, the *in vivo* significance of this multipotency is questionable and furthermore the *in vitro* experiments do not exclude heterogeneity within the tested precursor populations.

Several years ago we have described a small bone marrow derived precursor population with lymphoid and myeloid developmental potential *in vitro* (Balciunaite G, Ceredig R, Massa S, Rolink AG. Eur J Immunol. 2005 Jul;35(7):2019-30). This population comprises 0.1 – 0.3% of total BM nucleated cells and was characterized as being B220+ c-Kit+ CD19- NK1.1-. Now, by identifying new markers and by analyzing newly generated mutant mice we have found that this population can be subdivided into at least 6 subpopulations. The main marker we used for this was Ly6D. Single cell RNA- sequence analysis performed on the Ly6D+ and Ly6D-, B220+ c-Kit+ CD19- NK1.1- cells revealed within the Ly6D+ cells a largely lymphoid restricted transcriptome whereas the Ly6D- cells showed either a transcriptome reminiscent of lymphoid, myeloid or dendritic cells. Thus these findings reveal that B220+ c-Kit+ CD19- NK1.1- cells are very heterogeneous and that myeloid and lymphoid developmental potential is coming from different cells within this population.

Selected Publications

Gehre N, Nusser A, von Muenchow L, Tussiwand R, Engdahl C, Capoferri G, Bosco N, Ceredig R, Rolink AG. (2015) A stromal cell free culture system generates mouse pro-T cells that can reconstitute T- cell compartments *in vivo*. European journal of immunology 45, 932–942

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Tsapogas P, Swee LK, Nusser A, Nuber N, Kreuzaler M, Capoferri G, Rolink H, Ceredig R, Rolink A. (2014) *In vivo* evidence for an instructive role of fms-like tyrosine kinase-3 (FLT3) ligand in hematopoietic development. Haematologica 99, 638–646

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Connection to Clinical Practice

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The prophylactic and/or therapeutic effects of IL-2 – anti - IL-2 complexes on SLE like cGVHD

cGVHD as can be induced by the injection of DBA/2 T cells into semi-allogeneic (C57/Bl6 x DBA/2) F1 (BDF1) mice shows many similarities to SLE in man. Some years ago it was shown that injection of IL-2 anti-IL2 complexes into normal mice can result into a dramatic increase of regulatory T cells (Tregs) or CD8 T cells. The increase of either Tregs or CD8 T cells was dependent on the anti-IL2 antibody that was used for generating the complexes. We have now tested the prophylactic and therapeutic effects of these complexes on SLE like cGVHD. Our findings indicate that the treatment of BDF1 mice before the injection of DBA/2 T cells with complexes that induce a Treg increase to a large extent prevents the development of cGVHD whereas the injection of the complexes that induce a CD8 increase had no effect or even made the disease worse.

In marked contrast no amelioration of the cGVHD was observed when IL-2 complexes that induce Treg cells were given 3 weeks after induction of a cGVHD (therapeutic protocol). However, the therapeutic treatment that induces a CD8 increase very significantly ameliorated the autoimmunity as determined by the titer of autoantibodies and the development of immune complex glomerulonephritis. No improvement of disease by these complexes was observed when the cGVHD was induced by CD8 depleted DBA/2 T cells. This finding suggest that the ameliorating effects by the therapeutic treatment with CD8 inducing complexes might be due to an increase of DBA/2 CD8 T cells.

Overall, these studies indicate that IL-2 complexes might well be envisaged for prophylactic and/or therapeutic therapies of cGVHD and may be even SLE.

Immuno-regulation

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Regulatory T cells profit from IL-7R signaling during peripheral and allograft tolerance

In mammals, regulatory T cells (Tregs) establish and maintain immune responses toward self- and non–self-antigens by suppression of specific T cells.
The population of Tregs consists of functionally different Treg subsets that regulate different immune responses. Although many studies investigated the function of Tregs, the mechanisms controlling homeostasis and activation of different Treg subsets are poorly defined.
We identified that IL-7 signaling, beside its known role in survival, stabilizes the function of Treg in the lymph nodes (LN) during inflammatory T-cell responses. Treg prevented early and increased late IL-7 induction in lymph node stromal cells. Our data suggest that Treg require IL-7R signaling to increase their CD25 expression (Fig. 1) and efficiently compete with other T-cell subsets for IL-2. Improved IL-2 sensitivity stabilized the Treg phenotype and promoted expansion of effector Tregs (eTreg) *in vivo*. The specific ablation of IL-7R specifically on Treg reduced their suppressive function, causing a decrease of eTregs during aging (Fig. 2, A) and mild symptoms of autoimmunity during aging (Fig. 2, B). In various allograft models including skin allograft transplantation, Treg were shown to control immune responses to foreign antigens. We established a transplantation model in which I-Abm12 skin allografts are accepted on Rag2–/– mice, and the function of polyclonal Treg and their specialized subsets can be investigated after cotransfer with I-Abm12–specific CD4+ T cells. Transfer of high Treg numbers established allograft tolerance, which could be reverted by specific Treg depletion. Treg-suppressed ABM expansion dependent on the initial ratio and IL-2 production of ABM increased their own and Treg numbers. Treg displayed an effector phenotype in the LN. Blockade of IL-7 or transfer of Treg from Il7ra-ΔTreg mice caused graft rejection in 40 % of tolerant mice (Fig. 3), which could be due to reduced survival in the LN. However, the ratio of Treg/ABM in rejecting mice was in favor of Treg, which usually correlated with allograft tolerance. Therefore, our results suggest that IL-7R signaling does not exclusively support Treg survival but also increases the suppressive activity of allo-activated Treg. Structural lymph node follicular reticular cells LN-FRC and lymphatic endothelial cells (LEC) are the main source of IL-7 in the LN and increase IL-7 production on inflammatory T-cell responses. Consistent with this view, allogeneic CD4+ T-cell activation and release of IFNγ increased IL-7 levels in LN-FRC at early time points, which massively declined thereafter in draining LN of transplanted Rag2–/– mice. Treg inhibited both IFNγ production and expansion of ABM, which correlated with an early reduction of IL-7 transcription in LN-FRC of allograft-tolerant mice. At later time points, the presence of Treg resulted in higher IL-7 transcription; the reasons for this are unclear, and further experiments need to be done to address this. However, Treg can influence the LN microenvironment to facilitate suppression of T cells, which would allow IL-2 consumption and suppression in close proximity of activated T cells, as well as help to maintain immunological tolerance.

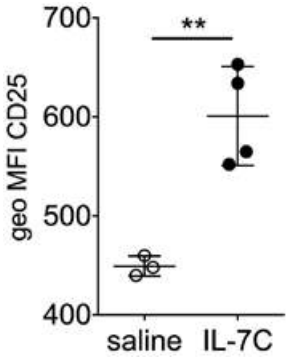

Fig. 1: CD25 expression is increased in presence of IL-7.
CD25 expression of Foxp3$^+$ T_{reg} isolated from a pool of brachial, axillary and inguinal lymph nodes of *Foxp3*-eGFP mice 24h after one single injection of IL-7 complex (IL-7C).

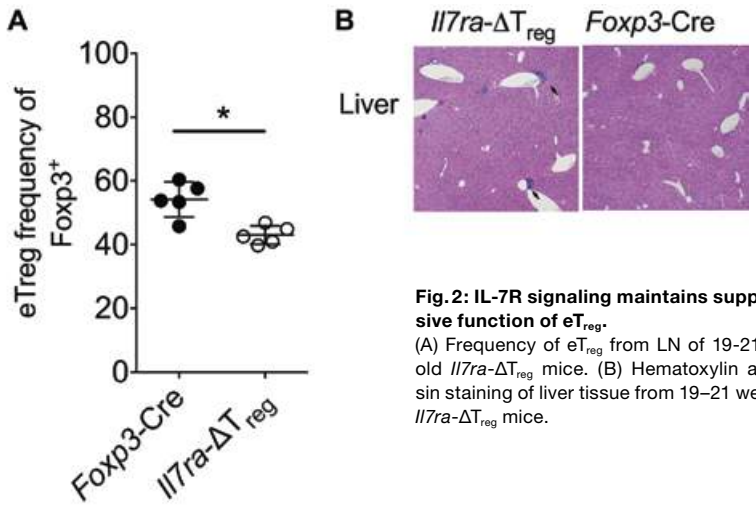

Fig. 2: IL-7R signaling maintains suppressive function of eT_{reg}.
(A) Frequency of eT_{reg} from LN of 19-21 week old *Il7ra*-ΔT_{reg} mice. (B) Hematoxylin and eosin staining of liver tissue from 19–21 week old *Il7ra*-ΔT_{reg} mice.

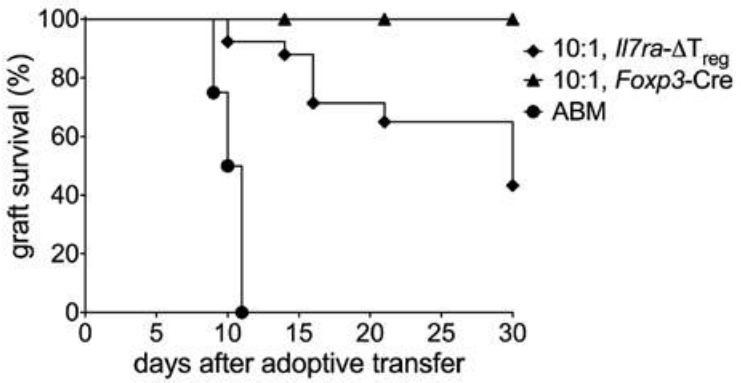

Fig. 3: Graft survival in presence of *Il7ra*-ΔT_{reg} Treg cells.
I-A^{bm12} tail skin transplanted Rag2$^{-/-}$ mice adoptively transferred with monoclonal transgenic anti I-A^{bm12} CD4$^+$ T cells (ABM) alone and different ratios of polyclonal Foxp3$^+$ cells:ABM. Percentage of graft survival for transplanted mice after adoptive transfer of ABM and Foxp3$^+$ T_{reg} from *Foxp3*-Cre and *IL7ra*-ΔT_{reg} mice.

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Immuno-therapy

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Natural killer cells in the control of disease relapse and infection in transplanted patients

Natural killer (NK) cells are a subgroup of lymphocytes that – unlike B- and T-lymphocytes – do not possess rearranged surface receptors but instead are regulated by integration of signals derived from an array of activating and inhibitory receptors. While much progress has been made over the last 10 years in the characterization of NK cell surface receptors and their ligands, the function and ligands of several NK cell receptors are still unknown.

NK cells are of particular importance in patients under pharmacological immunosuppression, e.g. after solid organ or hematopoietic stem cell transplantation. These patients with compromised adaptive immunity are therefore predestined to study the role of natural killer cells in the control of malignant and viral transformation.

Our studies focus on one family of NK cell receptors termed Killer-cell Immunoglobulin-like receptors (KIR). KIR are transmembrane proteins and come in an inhibitory or activating flavor. While the function of inhibitory KIR is clear (providing NK cell tolerance through binding to HLA-class I) both function and ligands of activating KIR are so far undefined. Studies in transplanted patients have hinted that patients carrying activating KIR have a reduced rate of viral infection, pointing to viral proteins as potential activating KIR ligands. Through studies involving solid organ grafts performed in transplant centers in Switzerland reporting to the Swiss Transplant Cohort Study, we have identified activating KIR receptors as having a protective role regarding the occurrence of cytomegalovirus replication after transplantation. These studies are accompanied by *in vitro* experiments aiming to resolve receptor-ligand interactions relevant for this protective role of NK cells.

Another line of research is directed towards the identification of activating KIR receptors involved in the recognition of acute myeloid leukemia cells, where clinical studies have also documented a benefit for patients receiving allografts from a donor carrying such activating receptor genes.

A more recently established line of research analyzes how KIR are involved in the antibody dependent cellular cytotoxicity (ADCC), an important mode of action of therapeutically administered monoclonal antibodies. We could show that the superior efficacy of modern third-generation glycoengineered antibodies is partly due to their ability to overcome inhibitory signaling by KIR receptors.

Finally, in a translational arm of our research and in close collaboration with the Division of Hematology at the University Hospital, we are treating patients with cancers incurable by conventional chemotherapy with highly purified and *ex vivo* expanded NK cells with the aim to eradicate disease through a combined chemo-/immunotherapy approach.

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Clinical Immunology

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Antibodies against complement C1q in systemic lupus erythematosus: The link between complement C1q, apoptosis, EBV, and primary hemostasis

Systemic lupus erythematosus (SLE) is the archetype of a systemic autoimmune disease. However, the causes and pathogenic mechanisms of SLE are still not fully understood. A major hypothesis of the pathogenesis of SLE assumes that the disease is driven by a defective clearance of dead and dying (apoptotic) cells. In the context of an altered clearance, these apoptotic cells could become antigenic and initiate an autoimmune response. The complement system has been shown to play an important role in the clearance of apoptotic cells and the deficiency of one of the early components of the classical pathway of complement is strongly associated with the development of SLE. However, most SLE patients have no primary complement deficiency. In contrast, hypocomplementemia in SLE patients is a secondary event and most often associated with antibodies against the first component of the classical pathway of complement (C1q). As we and others have shown, autoantibodies against C1q (anti-C1q) strongly correlate with the overall disease activity in SLE patients, but in particular with renal flares. Our studies suggest that the occurrence of anti-C1q in SLE patients is necessary but not sufficient for the development of severe lupus nephritis. It is possible that anti-C1q interfere with the normal function of the complement system including the clearance of apoptotic cells. In fact, we could already show that anti-C1q specifically target C1q when bound to the surface of early apoptotic cells. We could also show that anti-C1q can activate the classical as well as the lectin pathway of complement and induce a proinflammatory phenotype in macrophages. In addition, we identified a major linear epitope on the C1q molecule targeted by anti-C1q. The dissection of its core amino acid sequence revealed a striking sequence homology with Ebstein Barr Virus suggesting cross-reactivity through molecular mimicry. Independently, studying the binding characteristics of anti-C1q led us to the analysis of the binding of von Willebrand factor to bound C1q. Interestingly, we could observe binding of vWF to bound C1q allowing consecutive aggregation of platelets. Thus, we could demonstrate a direct interaction between starter molecules of primary hemostasis and the classical pathway of complement system that might contribute to the pathogenic mechanisms in complement-mediated, inflammatory diseases. Currently, our group aims to further examine the origin, pathological role and clinical relevance of anti-C1q antibodies in a double approach based on experimental studies of C1q and anti-C1q and clinical studies of patients with SLE. In the experimental studies we want to understand i) the origin of anti-C1q, ii) the consequences of the binding of antiC1q, and iii) the immunological consequences of the binding of von Willebrand Factor (vWF) to bound C1q.

Connection to Clinical Practice

Marten Trendelenburg
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In our clinical studies we are analysing the role of anti-C1q as a biomarker in SLE patients and it's relation to previous Ebstein Barr Virus infection. In addition and thanks to the participation in the Swiss SLE Cohort Study (SSCS) we are also studying the role of other autoantibodies and serum cytokines in SLE. Independent from anti-C1q studies, we are studying the role of complement split products (i.e. activation parameters) and complement mannan-binding lectin (MBL) in clinical settings. MBL is strongly related to C1q and has been shown to play an important role in the defence against infectious agents. More recent studies suggest that MBL also binds to apoptotic cells and plays a pro-inflammatory role in experimental settings of ischaemia-reperfusion injury. The high frequency of functional MBL deficiency in the general population (about 25 %) predestines MBL for clinical studies investigating its role in human diseases.

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Immune Regulation

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Understanding Dendritic Cell Biology

Dendritic cells (DCs) are professional antigen presenting cells that play a key role in the immune system. Under steady state conditions immature DCs promote tolerance and induction of regulatory cells. Upon pathogen recognition, DCs undergo maturation and initiate the adaptive immune response by priming T cells. DCs dictate the type of adaptive response towards a particular pathogen by secreting different type of cytokines, maintaining an appropriate inflammatory milieu and recruiting specialized effector cells. Such a complex and coordinated response requires DC subset specialization, which is obtained through lineage as well as functional specificity, controlled by complex transcriptional networks. It is therefore essential to understand the transcriptional regulation of existing subsets through the characterization of their developmental cues, as well as identify the transcriptional signature required for the functional properties of each subset.

Defining lineage commitment

Development of DCs occurs in the bone marrow and requires a complex transcriptional lineage specification (fig. 1), which promotes the progression from an uncommitted self-renewing stem cell progenitor to a mature DC. Several transcription factors have been implicated in the development of DC. Some transcription factors impairing DC development also affect other hematopoietic lineages and multiple DC subsets, while some prevent development of a given DC subset. Collectively, the hierarchical requirement of each of them during DC development is still unclear. We could recently show how hetero-complexes of transcription factors are promoting lineage specification. We aim at defining the networks, which are intrinsically required for the establishment of lineage commitment within dendritic cell subsets. Moreover, the transcriptional identity of each subset, which encodes and ensures functional specificity, allowing for the initiation of the appropriate response to a particular pathogen or immunological insult, has to be characterized. Given the critical role of DCs in bridging the innate and the adaptive immunity there is increasing interest in understanding the transcriptional network underlying their development as well as their functional specialization.

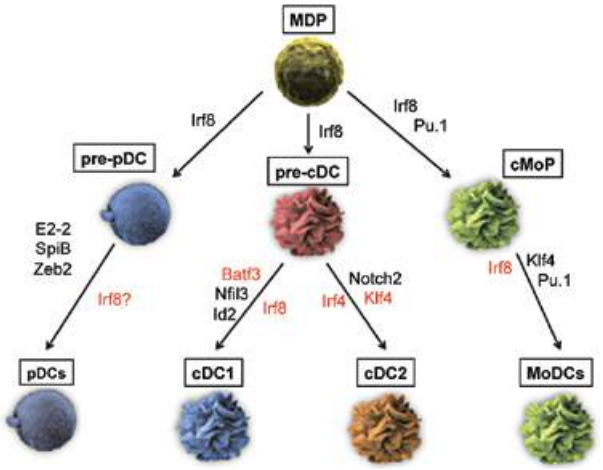

Fig. 1: Dendritic cell development
Dendritic cells develop in the bone marrow from a monocyte-Dendritic cell progenitor (MDP). Lineage commitment is determined by the acquisition of a specific transcriptional landscape at a determined stage of development. Extrinsic factors, proliferation rate and epigenetic changes will influence the expression of given transcription factors and determine the progression into a specific cell lineage, while restricting other fates.

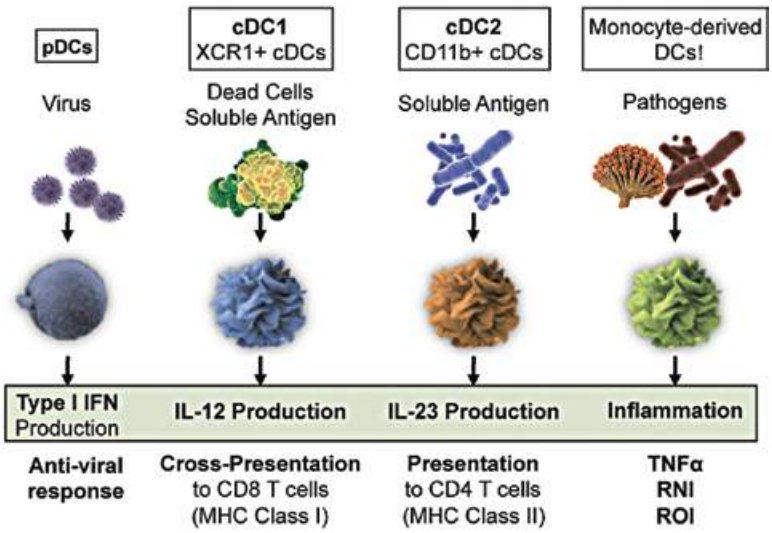

Fig. 2: Dendritic cell subsets
As depicted, we identify four major subsets of DCs. Each subset is able to efficiently recognize and respond to specific pathogens. Following recognition through danger and pattern associated recognition receptors (DAMPs; PAMPs), DC secrete specific cytokines and chemokines leading to the activation of the appropriate innate as well as adaptive immune response.

Deciphering the complexity of cDC2

DCs can be classified in three major branches based on their ontogeny: conventional DC (cDCs), plasmacytoid DCs (pDCs) and monocyte derived DCs. cDCs are further subdivided into cDC1 and cDC2 (fig. 2). cDC1 are a homogenous group, dependent on the transcription factors Irf8 and Batf3, and can be identified across all organs and tissues based on the expression of XCR1. On the contrary, cDC2 are highly heterogeneous, unified only by the expression of Irf4. The complexity of the cDC2 subset was further highlighted by single cell sequencing experiments. Transcriptional profiling of cDC progenitors allowed us to identify Klf4 as a transcription factor involved in the development of a subset of cDC2. A significant reduction of the cDC2 compartment is evident in the absence of the transcription factor Klf4. In some tissues the use of specific markers allowed us to unequivocally identify the Klf4-dependent cDC2 subpopulation, which in skin draining lymph nodes was previously recognized as double negative for the surface markers CD11b and CD24. Despite its identification, the role of this DC subset as well as the functional requirement for Klf4 was unknown. We could show that expression of Klf4 within cDCs is necessary for the induction of Type 2 immunity. Klf4 deficient mice are highly susceptible to parasitic infections, such as the helminthes *Schistosoma mansoni.* Further, impaired Th2 immunity in these mice shows increased resistance to the development of house dust mite induced asthma (fig. 3). The mechanisms underlying the pathogenesis of Th2 immunity are still unclear. The identification of the transcription factor as well as the DC subset involved in Th2 priming will be instrumental to understand how Th2 immunity is established, and potentially lead us to the development of therapeutic interventions.

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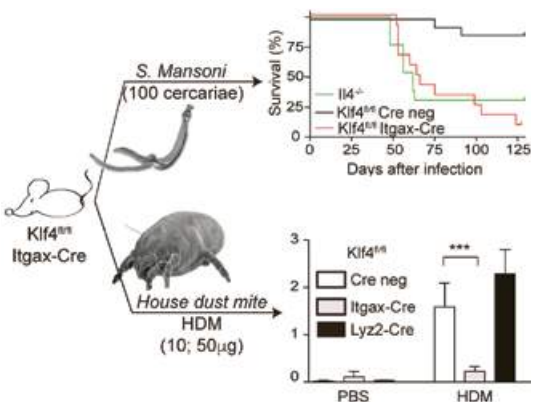

Fig. 3: Dendritic cell subsets
Expression of the transcription factor Klf4 within dendritic cells is required for the induction of Th2 immunity. Klf4 conditional-deficient mice are highly susceptible to *S. mansoni* infection and succumb around 50 days after infection, comparable to IL-4 deficient mice. Also the context of allergic reactions, Klf4 conditional-deficient mice show impaired Th2 immunity. This results in reduced eosinophil recruitment in bronchoalveolar lavage following intra-nasal challenge with house dust mite.

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