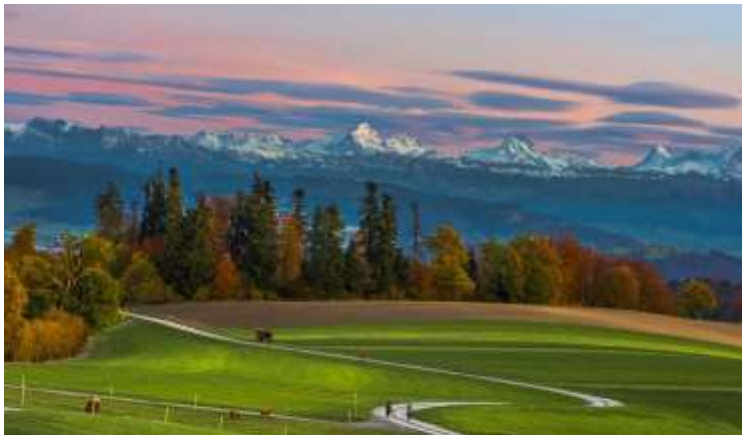
6th
SCRM PhD Student Retreat



Gurten Park
30 August 2019

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6th SCRM PhD Student Retreat

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30 August 2019

08:00	08:15	Welcome coffee
08:15	08:20	Welcome by the Organizing Committee
Morning Session Chair: Viviana Rubino		
08:20	08:40	Marcos Sande-Melon
08:40	09:00	Raphaela Seeger
09:00	09:20	Max Heydasch
09:20	09:40	Melle Holwerda
09:40	10:00	Annika Kratzel
10:00	10:20	**Coffee Break**
10:20	10:40	Yang Zhang
10:40	11:00	Patricia Renz
11:00	11:20	Chantal Bachmann
11:20	12:20	**Mentor Talk: Fiona Watt**
12:20	13:20	**Lunch Break**
Afternoon Session Chair: Carla Pernaci		
13:20	13:40	Jonathan Save
13:40	14:00	Cecilia Bazzini
14:20	14:40	Andrés Sanz Morejon
14:40	15:00	Yanyun Gao
15:00	15:20	**Coffee Break**
15:20	15:40	Viviana Rubino
15:40	16:00	Carla Pernaci
16:00	16:20	Mahmoud Hallal
16:20	17:20	**Mentor Talk: Caroline Blumer Toti**
17:20	17:30	**Conclusive Remarks and word of Thanks from the SCRM Steering Committee**
17:30		**Apéro**

This event was made possible with the generous support of:

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We want to thank you for your support, which made this event possible for the sixth time already.

1st Retreat, Münchenwiler Castle, 9th August, 2014

2nd Retreat. Gurten Mountain. 4th September 2015

3rd Retreat, Gurten Mountain, 6th September 2016

4th Retreat, Gurten Mountain, 1st September 2017

5th Retreat, Paul Klee Center, 31th August 2018

Dear participants,

Welcome to the 6th SCRM PhD Students Retreat!

We are happy to continue this successful history of seminars with you, which started in 2014, initiated by our colleague Dr. Luca Tamò.

The day starts with a welcome coffee and a short address given by the organizing committee. The program will continue with sessions of PhD project presentations and two coffee breaks for networking and discussions.

Then we are again looking forward to two interesting keynote lectures, which will be given by this year's mentors **Fiona Watt** from the King's College London, and **Caroline Blumer Toti** from Miltenyi Biotec. We are very grateful to them for being our mentors for a day.

The retreat will be finalized by an address given by the SCRM steering committee, followed by the Aperitif.

We are looking forward to meeting you and we wish you a fruitful and pleasant time during the retreat.

Sincerely yours,
The organizing committee

Viviana Rubino
Carla Pernaci
Mahmoud Hallal
Silvan Heeb

Marcos Sande-Melon

Pre-existent adult sox10+ cardiomyocytes contribute to myocardial regeneration in the zebrafish

During heart regeneration in the zebrafish, fibrotic tissue is replaced by newly formed cardiomyocytes derived from pre-existing ones. It is unclear whether the heart is comprised of several cardiomyocyte populations bearing different capacity to replace lost myocardium. Here, using sox10 genetic fate mapping, we identified a subset of pre-existent cardiomyocytes in the adult zebrafish heart with a distinct gene expression profile that expanded massively after cryoinjury. Genetic ablation of sox10+ cardiomyocytes severely impaired cardiac regeneration revealing that they play a crucial role for heart regeneration.

- Fiona Watt, King's College London
- Caroline Blumer Toti, Miltenyi Biotec GmbH

Additional Participants

- Constanze Raltschev, PhD student
- Xingshuo Zhang, PhD student
- Kristina Krempaska, PhD student

Guests

The SCRM Steering Committee:

- Prof. Eliane Müller
- Prof. Volker Enzmann
- Prof. Daniel Surbek
- Prof. Benjamin Gantenbein
- Dr. Amiq Gazdhar
- PD Dr. Carsten Riether
- Prof. em. Thomas Krause

- Personal Investigators of the PhD students
- Dr. William Hariton and Dr. Felix Baier
- Rene Aeberhard

Raphaela Seeger

Investigating synaptic vesicle exocytosis by structural studies of rat synaptosomes

Understanding how our brain works requires a comprehensive description of the molecular mechanism of vesicle exocytosis which allows inter-neural communication through neurotransmitter release.

The proteins involved in the vesicle exocytosis have been identified and the ultrastructure of synapses is resolved, however the protein structures have not yet been mapped in the images of the synapses and their roles in the mechanisms are often unclear. The aim is to acquire near atomic maps of synapses using Tomography and locating the components involved in vesicle exocytosis in order to get insights into their influences on the exocytosis mechanism. One main component is the network of filaments interconnecting the synaptic vesicles. They not only regulate the synaptic vesicle release activity but also is subject to rapid modification in the event of sustained activity.

An example for a component which is believed to be associated to the filaments connecting the vesicles with the plasma membrane, that would benefit from such a mapping is synaptotagmin. It is debated whether synaptotagmin is involved in vesicle insertion into the membrane as a calcium sensor, a map identifying its position would shine light onto this situation (O'Connor et al, 2002).

An effective method to visualize vesicle exocytosis is Cryo-electron Tomography (cryo-ET). Our established method of sample preparation enables us to not only resolve the environment of the synapse structurally, but also temporal with an accuracy of a few miliseconds. To analyze the obtained data, the new template matching that allows to localize a known 3D structure in a crowded molecular environment (Rickgauer et al, 2017) and the growing implementation of machine-learning frameworks into image reconstruction in cryo ET will be used (Waller et al, 2015).

Mahmoud Hallal

Characterization of Bypassing Kinases Inferred by Phosphoproteomics in a Midostaurin Resistant Myeloid Cell Line Model

Background: Chronic myeloid neoplasms are heterogeneous malignancies caused by sequential accumulation of genetic lesions in hematopoietic stem cells with a tendency to evolve towards acute myeloid leukemia. Reliable biomarkers to identify patients' chances for response to targeting compounds remain a crucial necessity in the current post-genomic era of precision medicine. We hypothesize that differential phosphoproteomic (PP) profiles might represent a suitable functional biological layer that allows to identify relevant determinants of oncogenic phenotypes.

Aim: The aim of the project was to build a bioinformatics pipeline using PP data to i) identify differentially phosphorylated sites, ii) infer targetable kinases and iii) characterize involved oncogenic pathways. Here, we present results from further exploratory experiments of a previously established PP analysis pipeline that enables to infer sensitive/bypassing kinase activities in a Midostaurin/PKC412 (MIDO) resistant myeloid cell line model.

Methods: For the current biological study, we applied our pipeline to explore MIDO resistance mechanism in published MIDO-resistant (rMOLM13) and sensitive (sMOLM13) cell lines. r/sMOLM13 were cultured in triplicates for 24hrs and subsequently exposed for 1h to 25nM MIDO or DMSO (CTRL). PPs were enriched with titanium-dioxide and analyzed by mass spectrometry (nanoLC-MS2). Our previously validated Kinase Activity Enrichment Analysis (KAEA) bioinformatics pipeline was further applied to infer kinase activities based on the SetRank enrichment algorithm.

Results: In the current biological exploration, we compared rMOLM13 vs sMOLM13 exposed to MIDO and CTRL. rMOLM13 showed slower proliferation and vacuolization compared to sMOLM13. 13,682 phosphorylation sites were identified in the four conditions rMOLM13_MIDO, rMOLM13_CTRL, sMOLM13_CTRL and sMOLM13_MIDO. We identified distinct clusters of over- or underexpressed sites associated with distinct conditions. We performed a KAEA comparison of rMOLM13_MIDO vs sMOLM13_MIDO and rMOLM13_CTRL vs sMOLM13_CTRL. CDK1 and other CDKs enriched as underactive in rMOLM13, in line with the observed reduced proliferation. Most interestingly, kinases PDHK1, PDHK2 and PKM, all pyruvate kinases involved in regulation of mitochondrial metabolism, enriched as overactive, as well as MAPK1 and CK2. Reduced proliferation, vacuolization and patterns of enriched kinases pointed towards autophagy as potential resistance mechanism in rMOLM13. We performed preliminary experiments with autophagy inhibitors in combination with MIDO, which supported a synergistic activity.

Conclusions: With our PP analysis pipeline, we identified biologically relevant mechanisms of resistance that will be explored as potential targets for combination therapy. They allow us to move forward to primary patient samples, which will eventually promote precision medicine based on functional biomarkers in patients with myeloid neoplasms.

Carla Pernaci

Investigating the role of cerebellar circuitry in Spinocerebellar ataxia-1 (SCA1) pathology

Spinocerebellar ataxia type 1 (SCA1), a devastating neurodegenerative disease (ND), caused by a polyglutammine (CAG) repeat expansion within the ubiquitously expressed Ataxin-1 (ATXN1) protein. Despite the expression of mutant ATXN1 from birth on, its toxicity and related pathological consequences are observed only in patient's mid-thirties, suggesting the presence of compensatory / adaptive responses that may precede and delay the pathology. During the disease course, the optimal functioning of cerebellar circuit is strongly compromised, affecting the physiological activity of Purkinje cells (PCs), finally driving their degeneration in the end stage of the disease. Here, we investigated early molecular changes that may underlie selective alteration in cerebellar circuitry thereby affecting PCs functioning. Using ATXN1[154Q/2Q] transgenic mice model, we focused on the major excitatory inputs onto PCs; the climbing fibers (CF), that has been shown to be structurally and functionally altered early on. We employed immunohistochemistry technique to investigate the development and the refinement of these synaptic connections during the first weeks of postnatal brain development. Indeed, we observed a strong delay in the synaptic expression of molecules such as mGlur1, TrkB , Cav2.1, crucial in the maturation and elimination processes of the weaker CF innervating PC dendrites. These synaptic changes has been observed together with a strong reduction in PC-associated mTORC1 signaling. Taking into account the critical role of mTORC1 signaling in protein synthesis, we hypothesized that diminished mTORC1 activation may be responsible of the delayed synaptic expression of the examined molecules. This molecular delay eventually leads to an impaired maturation of the excitatory component of the circuit, resulting in an abnormally excess of CF synaptic terminals innervating PCs. Since balance between neuronal Excitation/inhibition (E/I) is tightly regulated, focusing onto Parvalbumin+ expressing interneurons, we are investiganting how those early excitatory impairments may influence and perturb neuronal E/I balance. By pharmacogenetic modulation of neuronal activity, we aim to specifically identify activity-dependent molecular signatures in the affected PCs, validating them as downstream effectors of SCA1 pathology with the final goal to restore E/I balance within cerebellar circuit, thereby ameliorating the pathology.

Max Heydasch

Dissecting a Rho GTPase spatio-temporal signalling network regulating growth cone motility, neurite and axonal outgrowth

Rho GTPases are key signalling molecules for the spatio-temporal control of cytoskeletal dynamics. According to the classical models three canonical Rho GTPases regulate specific cytoskeletal structures such as filopodia (Cdc42), lamellipodia (Rac1) and stress fibres (RhoA). For neuronal cells this lead to a classical model, in which RhoA activity regulates growth cone collapse, whereas Rac1 and Cdc42 signalling is required for growth cone protrusion. However, the use of Fluorescent Resonance Energy Transfer (FRET) probes has revealed that RhoA is active at the tip of filopodia during growth cone advance. Knockdown of two RhoA specific GTPase activating proteins (GAPs), ARHGAP5 and DLC1, has led to different opposing growth cone phenotypes and different spatial activity patterns of RhoA. These findings led to the hypothesis that GEFs and GAPs do work in concert to finely regulate the activity of Rho GTPases within specific spatio-temporal domains. To elucidate the molecular players that control the Rho GTPase activation patterns, we are employing computer vision guided analysis of high resolution time-lapse images of individual growth cones in which candidate proteins have been knocked down. At the same time, we will observe how these knockdowns affect Rho GTPase activity with the help of FRET sensors. With this approach we hope to gain detailed insides into the molecular mechanisms of cytoskeletal dynamics and identify the molecular players that can be targeted to improve growth cone stability and enhance neurite outgrowth.

Melle Holwerda

Determining the replication kinetics and cellular tropism of the ruminant-associated Influenza D virus on human derived airway epithelial cells.

Influenza D virus (IDV) is the newest genus in the Orthomyxoviridae virus family and was first observed among swine with influenza-like symptoms in 2011. IDV-directed antibodies have been detected in a broad range of livestock animals like camelids, cattle and small ruminants, revealing a wide host tropism. Interestingly, IDV-directed antibodies are also observed in humans, albeit only in those with occupational exposure to livestock. Therefore, to analyze if IDV has a zoonotic potential towards humans, we inoculated well-differentiated primary human airway epithelial cell (hAEC) cultures from three biological donors with the D/Bovine/Oklahoma/660/2013 strain at 33°C and 37°C. In addition, we sequentially passaged IDV further on naïve hAEC cultures to determine whether infectious progeny virus is produced. We monitored the production and secretion of viral progeny using quantitative real-time PCR and virus titration. This revealed that IDV is able to efficiently replicate in hAEC cultures and can be sequentially passaged at both 33°C and 37°C. Moreover, due to the similarity of IDV with the human-associated Influenza C virus (ICV), we compared the viral kinetics and cell tropism of both viruses. This shows that both viruses have similar replication kinetics and share a cell tropism preference towards ciliated cells. Collectively, these results might indicate why humans with occupational exposure to livestock develop IDV-directed antibodies and might provide more insight into the zoonotic potential of IDV.

Viviana Rubino

IL-21 secreted by CD4 T cells reduces leukemia stem cell function in human and murine acute myeloid leukemia

Leukemia stem cells (LSCs) are resistant to standard treatment and to elimination by the immune system. Consequently, they represent the main reason for treatment failure and disease relapse. The IL-21 receptor (IL-21R) forms a heterodimeric complex with the common γ chain and is widely expressed by hematopoietic cell populations. Upon binding of its ligand interleukin 21 (IL-21), it modulates function of lymphoid and myeloid cells. The role of IL-21/IL-21R signalling in leukemia and LSCs is unknown.

In this study, we show that IL-21R is heterogeneously expressed on leukemic stem/progenitor cells (LSPCs) from newly diagnosed acute myeloid leukemia (AML) patients and that IL-21 is exclusively expressed by CD4 T cells in AML. IL-21 was significantly increased in serum of AML patients compared to healthy controls (median: 45.9 vs 1.3 pg/mL) and was identified as an independent positive prognostic marker for overall survival. Functionally, IL-21 significantly reduced colony-forming and re-plating capacity of primary LSPCs ex vivo. Importantly, hematopoietic stem/progenitor cells from healthy bone marrow (BM) donors did not express IL-21R and were unaffected by IL-21 treatment.

Based on these findings, we hypothesized that IL-21/IL-21R signalling restricts the functionality of AML LSCs. We used a well-established syngeneic murine AML model to study how IL-21/IL-21R signalling regulates LSCs function in vivo. IL-21R -proficient and -deficient lineage- Sca-1+ c-kit+ cells were transduced with three different AML oncogenes (MLL-AF9, MLL-ENL and BCR-ABL1/NUP98-HOXA9) followed by transplantation into immuno-competent IL-21R-proficient recipient mice. Similar to our findings in human AML, murine AML LSCs expressed IL-21R and IL-21 was exclusively secreted by conventional CD4 T cells in the BM but not in the blood of AML mice. IL-21R deficiency on LSCs resulted in a faster disease progression and reduced survival in all three AML models. Genetic blockade of IL-21/IL-21R signalling in AML increased LSCs number in the bone marrow of AML mice after leukemia transplantation, as analysed by colony assay and in secondary transplantation experiments.

Similarly, treatment of human AML LSCs with recombinant IL-21 reduced the frequency of human AML LSCs in patient-derived xenografts.

In summary, IL-21 secreted by CD4 T cells regulates LSCs function in vivo and contributes to leukemia control. Further investigations will focus on the underlying cellular and molecular mechanisms.

Yanyun Gao

Pemetrexed/cisplatin therapy increases cytidine deaminase (CDA) and thymidine phosphorylase (TYMP) expression thereby inducing sensitivity to 5'-deoxy-5-fluorocytidine (5'-DFCR) in Non-Small-Cell lung cancer cells

Background: Lung cancer is the most common cause of cancer-related deaths in developed nations. More than 80% of lung tumors are non-small-cell lung cancers (NSCLC). Cisplatin plus pemetrexed (MTA) combination therapy is considered the standard treatment for patients with advanced non-squamous NSCLC. However, advanced NSCLC has a 5-year survival rate of below 10%, which is mainly due to therapy resistance. We previously showed that the NSCLC cell line A549 harbors different subpopulations including a mesenchymal-like subpopulation para clones featuring increased chemo- and radiotherapy resistance. Besides, the ratio of para clones increased after schedule-dependent MTA and cisplatin chemotherapy. Cytidine deaminase (CDA) and thymidine phosphorylase (TYMP) were important enzymes in pyrimidine salvage pathway, which is important for cancer cells responding to chemotherapy. The aim of this study was to investigate how CDA and TYMP associate chemotherapy resistance and how to eradicate resistant lung cancer cells.

Methods: Cell viability APH assay and colony formation assay were used to select drugs which targets mesenchymal chemoresistant A549 subpopulations. Western blot was used to test CDA and TYMP expression change after chemotherapy treatment in different NSCLC cell lines. Cell growth curve was used to evaluate the efficacy of sequential combination, first MTA and cisplatin, then 5'-DFCR. Immunohistochemistry (IHC) was used to test if CDA and TYMP expression increase after chemotherapy treatment in mice.

Results: High expression of CDA and TYMP associated with MTA and cisplatin chemotherapy resistance and sensitivity to 5'-DFCR. CDA and TYMP were highly expressed in mesenchymal-like A549 subpopulation para clones, which were chemotherapy resistant. High expression of CDA and TYMP was related to poor survival for the patients after chemotherapy. Para clones were sensitive to 5'-deoxy-5-fluorocytidine (5'-DFCR), comparing with holo clones in A549 because of high expression of CDA and TYMP. Interestingly, chemotherapy treatment increased CDA and TYMP expression during recovery phase in lung cancer cells. Besides, increased expression of CDA and TYMP induced by chemotherapy treatment was more pronounced in Kras mutant NSCLC cells including A549, H358 and H441 cell lines. Addition of 5'-DFCR at recovery day 2 after schedule dependent MTA and cisplatin treatment was most efficient treatment to eradicate chemotherapy resistant NSCLC cells. Based on the IHC images, we didn't find the CDA expression increase after MTA and cisplatin chemotherapy treatment in mice comparing with the untreated group.

Annika Kratzel

Susceptibility of human and camelid airway epithelium to coronaviruses

The Middle East Respiratory Syndrome Coronavirus (MERS-CoV) is a zoonotic virus that is transmitted from dromedary camels to humans. A close genetic relation to bat coronaviruses suggests that MERS-CoV may actually originate from MERS-like bat coronaviruses. Interestingly, the human coronavirus 229E (HCoV-229E) appears to have a similar history of cross-species transmission, since close relatives were detected in bats as well as in camelid species, including dromedary camels.

As a first step to identify viral and host factors involved in coronavirus cross-species transmission and host adaptation we characterized the viral replication kinetics of MERS-CoV, HCoV-229E and camel 229E-like viruses in human and llama camel airway epithelial cell (AEC) cultures. For MERS-CoV we observed robust replication kinetics in both the human and llama AEC cultures. In contrast, HCoV-229E only replicated efficiently in human AEC cultures, whereas replication of the genetically related 229E-like camel coronavirus was restricted to camelid AEC cultures. In addition to the replication kinetics, we assessed the cell tropism of MERS-CoV, HCoV-229E and camel 229E-like viruses via microscopic immunofluorescence analysis over time. Interestingly, both the camel 229E-like virus and MERS-CoV display a predominant affinity for ciliated cells in the llama AEC cultures, whereas in human AEC cultures MERS-CoV predominantly infects non-ciliated cells similar to HCoV-229E.

These results indicate that there are species-specific differences in the cell tropism and permissiveness for HCoV-229E and the camel 229E-like virus that limit cross-species transmission, whereas for MERS-CoV this is not the case. Further studies on the identification of viral and host factors involved in cross-species transmission will be necessary to better understand the emergence of coronavirus into the human population.

Yang Zhang

Identifying new drug targets to promote chemo- and FGFR-targeted therapies in squamous cell lung carcinoma.

Squamous cell lung carcinoma (SQLC) is a common type of lung cancer, causing approximately 400,000 deaths per year worldwide. No targeted therapies have been approved to date for SQLC. Instead, the standard of care for first-line palliative systemic therapy remains platinum-based doublet chemotherapy, a clinical scenario that has not changed considerably for nearly two decades .

DNA sequencing studies have revealed genomic alterations in fibroblast growth factor receptor (FGFR) family, including amplification of FGFR1, recurrent activating mutations of FGFR2 and FGFR3, and FGFR1/3 fusions, making FGFR the biggest class of "druggable" targets in SQLC. Although FGFR1 amplifications were associated with sensitivity to FGFR inhibition in preclinical models of SQLC , clinical trials with FGFR inhibitors (BGJ398, AZD4547 and JNJ-42756493) experienced a partial response, only 11% of patients with FGFR1-amplified SQLC responded. These observations support that FGFR1 amplification associates with FGFR dependency in some cases, but also suggest insufficient tumor cell killing by FGFR inhibition alone. Understanding molecular determinants that limit FGFR-targeted therapy may guide the development of novel strategies to more effectively treat FGFR1-amplified SQLC.

CRISPR-mediated genome editing provides a rapid and simple genetic system with which to identify novel drug targets in cancer. The key components of CRISPR include a guide RNA (sgRNA) and Cas9 nuclease. Base pairing between a sgRNA and target sequence directs cleavage of the target DNA by Cas9. DNA breaks generated by CRISPR can be repaired imprecisely, resulting in small insertions or deletions that inactivate the target gene. In this study, we will perform CRISPR screens to identify genetic determinants that limit efficacy of chemotherapy and FGFR-targeted therapy in SQLC. First, we will perform CRISPR screen using a custom sgRNA library for “druggable” genes with available small-molecule inhibitors. Second, we will validate candidate drug target genes and test the efficacy of inhibitors in vitro and in preclinical mouse models.

Andrés Sanz Morejon

Wilms Tumor 1b Expression Defines a Pro-regenerative Macrophage Subtype and Is Required for Organ Regeneration in the Zebrafish

Organ regeneration is preceded by the recruitment of innate immune cells, which play an active role during repair and regrowth. Here, we studied macrophage subtypes during organ regeneration in the zebrafish, an animal model with a high regenerative capacity. We identified a macrophage subpopulation expressing Wilms tumor 1b (wt1b), which accumulates within regenerating tissues. This wt1b+macrophage population exhibited an overall pro-regenerative gene expression profile and different migratory behavior compared to the remainder of the macrophages. Functional studies showed that wt1b regulates macrophage migration and retention at the injury area. Furthermore, wt1b-null mutant zebrafish presented signs of impaired macrophage differentiation, delayed fin growth upon caudal fin amputation, and reduced cardiomyocyte proliferation following cardiac injury that correlated with altered macrophage recruitment to the regenerating areas. We describe a pro-regenerative macrophage subtype in the zebrafish and a role for wt1b in organ regeneration.

Cecilia Bazzini

Characterization of anti-alpha 2 macroglobulin-like 1 autoantibodies in Paraneoplastic pemphigus.

Paraneoplastic pemphigus (PNP) is a rare and severe variant of pemphigus associated with an underlying malignancy and poor therapeutic outcome. PNP is characterized by the presence of autoantibodies against a broad spectrum of desmosomal components, additionally more than 50% of patients also have antibodies against the extracellular protease inhibitor alpha 2 macroglobulin-like 1 (A2ML1). A2ML1 expression in the epidermis is restricted to the granular layer and its biological function is still unknown.

The detection of anti-A2ML1 antibodies is presently based on immunoprecipitation of differentiated human keratinocyte extracts followed by polyacrylamide gel autoradiography or immunoblotting. Faster and more reliable diagnostic tools are thus needed. Our method relies on the expression of full-length A2ML1 in fusion with enhanced green fluorescent protein (EGFP). The secreted EGFP-A2ML1 and EGFP, as negative control, were expressed in transfected human embryonic kidney 293T cells and immunoprecipitated from the culture medium with PNP A2ML1-negative or -positive sera. The measurement of fluorescence in immunoprecipitates correlates with the results obtained with current methods. Moreover, we developed an ELISA test by immobilizing EGFP-A2ML1 and EGFP on 96-well plates. With this method and the presently set cutoff value, 42 % of the tested PNP sera (n=36) were positive. Among them, 25 % were strongly positive while none of the sera of pemphigus vulgaris (n=20) and bullous pemphigoid patients (n=20) were positive.

These assays were also useful for the screening of immortalized memory B cells from PBMCs isolated from a PNP patient, whose serum was positive for anti-A2ML1 antibodies, with the aim to isolate human monoclonal PNP Abs reacting with A2ML1 and test their effects on cell-cell adhesion.

Furthermore, we are going to start knocking down A2ML1 expression in organotypic cultures to investigate the potential function of A2ML1 in skin homeostasis.

All these findings will help to better define the phenotype(s) associated with anti-A2ML1 autoantibodies, thereby giving additional clues to the biological function of A2ML1.

Patricia Renz

Understanding astrocyte polarization in perinatal white matter injury and its contribution to disease outcomes

Introduction
White matter injury (WMI) is the most common form of brain injury in preterm infants. It is characterized by reactive microgliosis and astrocytosis, delayed oligodendrocyte differentiation, and in severe cases, neuronal death. So far, two different types of reactive astrocytes are recognized in brain injury, A1 astrocytes (A1s), which promote neurodegeneration and A2 astrocytes (A2s), which support neuronal survival and tissue repair. Given recent findings that A1 formation is induced by activated microglia and that these astrocytes delay oligodendrocyte differentiation and promote neuronal death, we hypothesize that A1s play a central role in WMI.

Materials and Methods
Several WMI rat models where tested. We used a combination of hypoxic-ischemic and inflammatory insults, a combination of fetal inflammation and postnatal hypoxia, and an only inflammatory insults. In situ hybridization with probes for A1–specific mRNA transcripts was performed on brain tissue from injured and control neonatal rat brains. Also, immunohistochemistry (IHC) was performed on injured and control postnatal day 11 brains.

Results
In situ hybridization experiments demonstrate a significant increase in the prevalence of A1 astrocytes in subcortical white matter tracts after WMI in our rodent models. IHC showed the severity of the WMI.

Conclusion
We demonstrate the formation of A1 reactive astrocytes in rodent models of WMI. This result is an important step towards understanding astrocyte polarization in WMI and opens the door to experiments investigating whether prevention of A1 formation ameliorates WMI disease outcomes.

Chantal Bachmann

Immune-Checkpoints in the Regulation of Leukemia and Cancer Stem Cells

Chronic myeloid leukemia (CML) shows many characteristics also present in its healthy counterpart, normal hematopoiesis: a rare (cancer) stem cell on top of the hierarchy, which divides only infrequently, shows self-renewal and can give rise to the different lineages present in the healthy tissue or, in case of CML, in the cancer. As chemotherapy mainly targets bulk cancer cells but spares the more resistant cancer stem cells (CSCs), CSC-targeting therapies are needed. Immune-Checkpoints like PD1, LAG3 or TIM3 are receptors regulating T-cell activation, but it has been shown that they can also be expressed on subsets of cancer cells in different types of cancer. For example, TIM3 is upregulated on AML CSCs where it drives their self-renewal via an autocrine loop with its ligand. To screen for immune checkpoint receptors and ligands of importance for CML and CML CSCs, we will set up a pooled small-scale in vivo CRISPR-KO screening. We are generating a small pooled library of single-guide RNAs targeting 25 different genes of interest, and clone it into a lentiviral vector to transduce isolated Cas9-expressing CML stem cells. The mixed cell population will then be used to generate a secondary CML in non-irradiated recipients. Positive and negative effects of KO can be assessed by measurement of the abundance of the respective sgRNAs by NGS. Effects of KO can also be analysed in vitro by colony formation. Once this system is established it will be a versatile tool to study different cancer models and genes.

Jonathan Save

Evaluation of phage therapy for the treatment of S. aureus endocarditis.

The problem of antibiotic resistance.
Staphylococcus aureus is a gram positive commensal bacteria that colonizes the nazopharingal cavity. A fundamental biological property of S. aureus is the ability to colonize healthy people. Approximately 30% of humans are persistent nasal carriers. The primary mode of transmission of S. aureus is by direct contact, usually skin-to-skin contact with a colonized or infected individual, although contact with contaminated objects and surfaces may also play a role. The use of antibiotics as a standard treatment is becoming problematic due to the spread of antibiotic-resistant strains such as methicillin-resistant S. aureus (MRSA) and vancomycin-resistant S. aureus (VRSA). The overall burden of staphylococcal diseases, particularly caused by MRSA, is increasing in many countries in both healthcare and community settings. The first wave of antibiotic resistance in S. aureus began in the mid-1940s following the massive use of penicillin. Introduction of methicillin in 1960 to fight against penicillin-resistant strains marked the begining of the second wave of resistance, just few months after its introduction on the market. Some years after, vancomycin was introduced to fight MRSA strain. Unfortunatly, in 2000 the first vancomycin-resistant strains has been reported. Skin and soft-tissue infections are the most common type of MRSA infection but some strains can also become especially virulent leading to infections of the central nervous system, bones, joints, lungs and heart (endocarditis).

Phage therapy.
To fight the growing burden of antibiotic resistance, bacteriophages (phages) represent an alternative for the control of S. aureus infections. Phages are viruses that specifically infect bacteria. They have coevolved with their hosts, thereby optimizing their spread and release mechanisms from the bacterial cell. Some phages are able to infect and kill some antibiotic resistant bacteria, such as the phage φMR11 against MRSA. Many studies have proven the effectiveness of phage therapy in diverse preclinical models of infection. Moreover, an increasing number of successful human treatments are reported, as for instance in digital osteomyelitis or urinary tract infection.

Goal of my PhD.
The main goal of my thesis is to evaluate phage therapy in a model of S. aureus infective endocarditis in rats.