**Medizinerkolleg Münster**

Am 05. und 06. Februar 2022

# Abschlusskolloquium der MedK Kohorte 2020_2

Liebe Teilnehmer*innen,

wir begrüßen Sie herzlich zum Abschlusskolloquium des Medizinerkollegs 2020_2.

Da wir das Kolloquium leider nicht als Präsenzveranstaltung, sondern als Zoom-Konferenz abhalten müssen, möchten wir Ihnen vorab ein paar Informationen zu diesem Format zukommen lassen:

Bei den Vorträgen ist eine Redezeit von 15min und Diskussionszeit von 5min vorgesehen. Sie sind alle herzlich eingeladen, sich aktiv an den Diskussionen zu beteiligen und gerne viele Fragen zu stellen. Wenn Sie gerade keinen Redebeitrag leisten möchten, bitten wir Sie das Mikrofon auszustellen.

Im Rahmen eines wertschätzenden Miteinanders während Zoom-Konferenzen möchten wir Sie bitten ihre **Kamera einzuschalten**, sofern dies für Sie technisch möglich ist.

Neben den Vorträgen wird es ein weiteres Präsentationsformat geben, welches die in den vergangenen Jahren durchgeführten Postersessions ersetzt. Diese **alternativen Postersessions** werden wie folgt ablaufen:

- Die Präsentierenden bekommen jeweils einen Breakoutraum zugeteilt und präsentieren Ihr Thema in einer 5minütigen Kurzpräsentation. Anschließend kann 10min diskutiert werden. Abstracts zu den Kurzpräsentationen finden Sie ab Seite 5 des Programmheftes
- Alle anderen Teilnehmer*innen können sich aussuchen, welchen Raum Sie besuchen und werden gebeten sich möglichst gut auf die 5 angebotenen Räume zu verteilen.
- Nach 15min werden die Breakouträume geschlossen und für eine weitere Runde erneut geöffnet. Sie haben die Möglichkeit 4 der 5 angebotenen Räume zu besuchen.

Wir wünschen allen Teilnehmer*innen ein spannendes und erfolgreiches Kolloquium,

Das Organisations-Team

Lisa Renate Richter (Kohortensprecherin 2020_2)
Jan Vorwerk (Kohortensprecher 2020_2)

Prof. Dr. Rupert Hallmann (Sprecher des MedK)
Melanie Wilbers (Studienkoordinatorin MedK)

## Programm 05.02.2022:

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| 8:30 | Begrüßung | Prof. Dr. Rupert Hallmann |
| **Vorträge (Chair: Rupert Hallmann)** | | |
| 8:40 | *"Receptor-interacting proteins in the regulation of the G protein-coupled chemokine receptor CCR10"*, Prof. Dr. Ursula Rescher, Institut für Medizinische Biochemie, ZMBE | Magdalena Thome |
| 9:00 | *"Functional characterization of FOXO1 in pediatric Burkitt lymphoma",* Prof. Birgit Burkhardt, Klinik für Päd. Hämatologie und Onkologie | Lea Kosch |
| 9:20 | *„SSEA-4 as a marker for tumorigenicity and metastasis in Ewing sarcoma"*, Prof. Dr. med. Claudia Rössig, Klinik für Kinder- und Jugendmedizin - Pädiatrische Hämatologie und Onkologie | Lisa Richter |
| 9:40 | *"The protective effect of glutamine on apoptosis and mitochondrial function in renal tubular epithelial cells upon acute kidney injury",* Prof. Dr. Alexander Zarbock, Klinik für Anästhesiologie, operative Intensivmedizin und Schmerztherapie | Corinna Lüneburg |
| 10:00 | **Pause** 15 min | |
| **Postersession I (Chair: Albrecht Schwab)** | | |
| 10:15 | **PSI-BR01**: *„Phase specific role of macrophages in colonic anastomotic healing"*, PD Dr. Felix Becker, Allgemein-, Viszeral- und Transplantationschirurgie | Maximiliane Winter |
| | **PSI-BR02**: "*Quantification of tumor characteristics with MRI for tumor characterization and therapy evaluation"*, Prof. Dr. Christoph Schliemann, Medizinische Klinik A | Emily Hoffmann |

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| | **PSI-BR03**: *"Development of a cell-derived matrix-based toolbox to study fibrosis in pancreatic cancer",* Prof. Dr. Albrecht Schwab, Institut für Physiologie II | Rieke Schleinhege |
| | **PSI-BR04**: *„The role of Glucocorticoids in Modulating the proinflammatory role of monocytes"*, Prof. Dr. Johannes Roth, Institut für Immunologie | Cornelia Niemeier |
| | **PSI-BR05:** *„The role of the KIBRA phosphorylation"* PD Dr. J. Kremerskothen,Medizinische Klinik D | Jana John |
| 11:15 | **Pause** 15 min | |

**Vorträge (Chair: Prof. Johannes Eble)**

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| 11:30 | *"Influence of TRPC cation channels on osteoclastogenesis"*, Prof. Dr. Richard Stange, Institut für Muskuloskelettale Medizin - Abteilung für Regenerative Muskuloskelettale Medizin | Leonie Caroline Cohaus |
| 11:50 | *"Cloning and expression of the Clec-2 ectodomain to isolate rhodocytin from snake venom"*, Prof. Dr Johannes Eble, Institut für Physiologische Chemie und Pathobiochemie | Mascha Feickert |
| 12:10 | "*Plasma activated water as an innovative disinfectant to inactivate waterborne planktonic microorganisms*", Prof. Dr. Thorsten Kuczius, Institut für Hygiene – Krankenhaus- und Umwelthygiene | Nahla Droste |
| 12:30 | **Mittagspause** 40 min | |

**Vorträge (Chair: Prof. Thomas Weide)**

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| 13:10 | *"Analyses of putative disease-associated Crumbs2 mutants"*, Prof. Dr. Thomas Weide, Molekulare Nephrologie - Medizinische Klinik D | Birgit Schönhoff |
| 13:30 | *“Immunofluorescence analyses aid diagnosis of Nephronophthisis“,* Prof. Dr. Martin Konrad - Pädiatrische Nephrologie | Carlotta Hellmann |
| 13:50 | *„Characterization and functional analysis of 'upstream open reading frame' (uORF)-encoded peptides and cancer-associated somatic uORF mutations.*”, PD Dr. Torsten Kessler, Dr. Dr. Klaus Wethmar, Medizinische Klinik A | Laura Szymik |
| 14:10 | *“ Subgroup-specific analysis of cellular metabolism in medulloblastoma“*, PD Dr. Kornelius Kerl, Klinik für Kinder- und Jugendmedizin - Pädiatrische Hämatologie und Onkologie | Viktoria Funke |
| 14:30 | **Pause** 15 min | |

**Postersession II (Chair: Prof. Barbara Kahl)**

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| 14:45 | **PSII-BR01**: *„Characterization of innate immune memory in phagocytes”*, Prof. Dr. Thomas Vogl, Institut für Immunologie | Jonas Wolf |
| | **PSII-BR02**: *“Influence of oncogene and non-coding RNA inactivation on lung cancer metastasis“,* Prof. Dr. Wolfgang Berdel, Medizinische Klinik A | Najeeb, Shammaa |
| | **PSII-BR03**: *„Characterization of S.aureus isolates with the same clonal background but different resistance oattern and mechanisms.“* Prof. Dr. Barbara Kahl, Medizin. Mikrobiologie | Antonia Roch |
| | **PSII-BR04**: *„Inhibiting PI3K-AKT-mTOR signaling in Multiple Myeloma-associated mesenchymal stem cells impedes the proliferation of Multiple Myeloma cells“* Prof. Dr. Cyrus Khandanpour, Med A | Luca Heinemann |

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| 15:45 | **Wrapup Tag 1** | Prof. Dr. Barbara Kahl |

## Programm 06.02.2022:

**Vorträge (Chair: Rupert Hallmann)**

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| 8:30 | *"The role of the GFI1-36N SNP in DNA double-strand break repair and cell cycle regulation as an approach to AML therapy",* Prof. Dr. Cyrus Khandanpour, Department of Medicine A | Jan Vorwerk |
| 8:50 | "*Impact of octeocyte-tumor cell crosstalk on bone homeostasis*", Prof. Dr. Thomas Pap Institut für Muskuloskelletale Medizin Arbeitsgruppe Osteoimmunologie | Tom Straukamp |
| 9:10 | "The role of Histone 2B phosphorylation in tumor pathogenesis" Prof. Dr. Michael Krahn, Med D | Sophia Zurhove |
| 9:30 | **Pause** 15 min | |

**Vorträge (Chair: Rupert Hallmann)**

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| 9.45 | *"Generation of a minicollagen-based, Neuropilin-1 specific and MMP14 activatable Trojan horse protein with a fluorescence effector domain"*, Prof. Dr. Johannes Eble, Institut für Physiologische Chemie und Pathobiochemie | Anna Duesmann |
| 10.05 | *"Localization and function of the cell adhesion molecule TMIGD2 in epithelial cells"*, Prof. Dr. Klaus Ebnet, Institut für molekulare Biochemie | Georg Karl Marin |
| 10.25 | *„Metabolomic regulation of acute kidney injury*", Prof.Dr. Jan Rossaint, Klinik für Anästhesiolgie, operative Intensivmedizin und Schmerztherapie | Robin Hettler |
| 10:45 | **Pause** 15 min | |

**Postersession III (Chair: Rupert Hallmann)**

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| 11.00 | **PSIII-BR01**: "*Characterization of the peripheral immune signature and functions of patients with relapsing-remitting multiple sclerosis treated with the immune reconstitution therapy Cladribine*", Prof. Dr. Luisa Klotz, Klinik für Neurologie mit Institut für Translationale Neurologie | Valerie Elisabeth Teschner |
| | **PSIII-BR02**: *"Scrutinizing the human TEX genes in the context of human male infertility"*, Prof. Dr. Frank Tüttelmann, Institut für Reproduktionsgenetik | Marie Helene Sieper |
| | **PSIII-BR03**: *"In vivo imaging of tumor-associated immune cell dynamics using time-lapse MRI"*, PD Dr. Carsten Höltke, TRIC | Ina Fredrich |
| | **PSIII-BR04**: *"Genetic polymorphisms in the promoter of the FSHB gebe and their impact on transcription factor binding"*, Prof. Jörg Gromoll, CeRA | Viktoria Wiebusch |
| | **PSIII-BR05**: "The action of follicular fluid on Slo3 channels from human sperm", Prof. Dr. Timo Strünker, CERA | Clara Eisenhardt |
| | **PSIII-BR06**: *"Detection of stress signals in E. coli using a fluorescent reporter gene-based system: Primary targets of different phytopharmaceuticals",* Prof. Dr. Ulrich Dobrindt, Institut für Hygiene | David Tutscher |
| 12:00 | **Mittagspause** 45 min | |

**Vorträge III (Chair: Rupert Hallmann)**

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| 12:45 | *"Influences of the small GTPase Rab7 on the expression of Thrombospondin Type-1 Domain-Containing 7A in podocyte injury"*, Prof. Dr. Hermann Pavenstädt, Institut für molekulare Nephrologie, Med D | Sofie Waimann |

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| 13.05 | *“fMRI in the Mouse Spinal Cord during peripheral somatosensory Stimulation*”, Prof. Dr. Cornelius Faber, Institut für klinische Radiologie | Amon Allelein |
| 13:25 | *„Establishing a model for mouse to human translation to explore the oncolytic potential of the Influenza A virus (IAV) PR8 in human lung tumor explants“,* Prof. Dr. Stephan Ludwig, Institut für molekulare Virologie | Julius Lange |
| 13:45 | **Rèsumè und Verabschiedung** | Prof. Dr. Rupert Hallmann |

## Poster-Abstracts:

### Postersession I:

**PSI-BR01*:* „Phase specific role of macrophages in colonic anastomotic healing“**

Maximiliane Winter

Priv.-Doz. Dr.Becker, Allgemein-, Viszeral- und Transplantationschirurgie

After surgical trauma, restoration of tissue integrity is the most crucial step in the complex process of intestinal anastomotic healing (AH). Intestinal AH is divided into three overlapping phases: an inflammatory phase, a proliferative phase, and a reparative phase. Each phase is defined and characterized by specific hallmark events and dynamic interactions between resident immune, endothelial, stroma and epithelial cells as well as infiltrated leukocytes. Macrophages play a decisive role in the process of AH, but their phase-specific function is not yet fully understood. The objective of our study was to analyze specific macrophage-elicited effects during the three phases of intestinal AH by using an animal model of colorectal anastomosis in combination with an innovative on demand monocyte/macrophage ablation strategy.

After establishing the model of colonic surgery and verifying local and systemic macrophage ablation, we investigated inflammatory infiltrate, angiogenesis, mucosal re- epithelialization, collagen synthesis and remodeling in phase-specific presence or absence of macrophages. We found histological evidence of a reduced inflammatory response, as well as reduced blood vessel ingrowth, in our macrophage-ablated mice. This results in better overall healing quality in the subsequent proliferative phase, with significantly advanced continuity and architecture of all colonic layers.

In summary, the results support our hypothesis that monocytes/macrophages elicit phase-specific effects during intestinal AH. Our data support that they have especially significant impact on anastomotic inflammation, resulting in advanced restoration of the colonic architecture. To better understand the effect of macrophage ablation on genetic expression, we will further investigate whole-transcriptome RNA sequencing and analysis.

**PSI-BR02**: **Quantification of tumor characteristics with MRI for tumor characterization and therapy evaluation**

Emily Hoffmann

Prof. Dr. Christoph Schliemann, Medizinische Klinik A

While the effects of classical cytotoxic chemotherapy can reliably be analyzed with measurement of lesion size in conventional CT or MR images, the assessment of therapy response to 'targeted therapies' is more challenging. Novel targeted therapies are designed to modulate specific characteristics of the tumor microenvironment and consequently do not have an immediate effect on tumor diameter, so innovative imaging approaches enable the quantitative assessment of specific tumor traits and their therapy-associated alterations.

A multiparametric MRI protocol comprising diffusion-weighted imaging (DWI), $T_1$ and $T_2$ mapping, oscillating gradient spin echo (OGSE), dynamic contrast-enhanced (DCE) and chemical exchange saturation transfer (CEST) MRI was used to assess treatment response to targeted therapies in a murine model of breast cancer. Tumor-bearing mice received either a treatment with immune checkpoint inhibitors (ICI, combination of anti-PD1 und anti-CTLA4) or the anti-angiogenic multikinase-inhibitor Sorafenib. After conducting the MRI scans, mice were sacrificed and the tumors prepared for an *ex vivo* validation of the assessed tumor features, including histology and immunohistochemistry.

The presented MRI protocol was able to characterize tumors and their therapy-induced changes regarding their heterogeneity, intratumoral hemorrhage and edema, endothelial permeability, immune-cell infiltrate and metabolism. The quantitative assessment of these characteristics revealed significant intratumoral changes after treatment with either ICI or Sorafenib, with extensive necrosis and increasing intratumoral hemorrhage over time in ICI-treated tumors, leading to an increase in size, and less intratumoral hemorrhage after treatment with sorafenib, due to stabilization of endothelial permeability.
The applied imaging protocol thus enables optimized response assessment of targeted therapies.

**PSI-BR03: “Development of a cell-derived matrix-based toolbox to study fibrosis in pancreatic cancer”**

Rieke Schleinhege

Univ.-Prof. Dr. med. Albrecht Schwab, Institut für Physiologie II

Fibrosis is a crucial feature of pancreatic pathologies such as chronic pancreatitis and pancreatic ductal adenocarcinoma (PDAC). In PDAC, fibrosis is associated with increased invasion of tumor cells and it hampers drug delivery. On the other hand, anti-fibrotic therapy accelerates PDAC progression. It is known that the bulk of the fibrotic extracellular matrix is produced by pancreatic stellate cells (PSCs), but it is yet not well understood how changes in the microenvironment affect matrix production. Thus, we aimed to get more insights into the mechanisms of fibrosis formation by developing an *in vitro* toolbox for PSC-derived matrices.

We induced matrix formation *in vitro* using the human PSC cell line PS-1. PSC-derived matrices were visualized and quantified with both Sirius Red-based and fluorescent imaging techniques. In addition, the matrix composition was analyzed using mass spectroscopy. To test whether pancreatic cancer cells interact with the matrix, we performed migration experiments with the human cell line Panc-1.

We found that PS-1 cells produce large amounts of extracellular matrix composed of multiple collagens and glycoproteins. Vitamin C greatly increases matrix synthesis of stimulated PS-1 cells. Pancreatic cancer cells migrate on this matrix in a directional manner which is in contrast to the migratory behavior on an artificially reconstituted matrix. We then decided to further address the mechanistic role of vitamin C in matrix formation. Using $Ca^{2+}$ imaging we showed that vitamin C induces a $Ca^{2+}$ influx into PSCs, partly mediated by the $Ca^{2+}$ channel Orai1.

In conclusion, we established a powerful toolbox to tackle various questions regarding pancreatic fibrosis and initiated further studies by describing the significance of vitamin C-dependent $Ca^{2+}$ signals in PSCs.

**PSI-BR04: „The role of Glucocorticoids in Modulating the proinflammatory role of monocytes“**

Cornelia Niemeier

Prof. Johannes Roth, Institut für Immunologie

Monocytes and macrophages are a central part of the innate immune system and play an important role not only in generation of the inflammatory response but also contribute to the resolution of inflammation. Many autoimmune and allergic diseases are linked to a dysfunctional or imbalanced immune system and glucocorticoids (GC) are still the most frequently used drugs for the treatment of these diseases. It is generally assumed that the beneficial effects of GC on monocytes are mainly due to suppression of their proinflammatory properties. However, the treatment of naïve monocytes with GC do not simply suppresses their proinflammatory function but rather induces differentiation of an anti-inflammatory and regulatory phenotype. However, the influence of GC on inflammatorily activated monocytes is still not well defined. Our group could show that stimulation of LPS-activated monoctyes with GC resulted in synergistic upregulation of specific group of genes that have not been affected by LPS or GC alone. Moreover, we identified the transcription factor FOXO3 as an master regulator of the LPS-GC induced phenotype in monocytes and AMPK as an upstream kinase modulating the Foxo3 activity in human monocytes. Applying the FOXO3 and AMPK inhibitor, Carbenoxolone, and Dorsomorphin respectively, we investigated the Foxo3-dependent regulation of gene expression. in LPS-induced proinflammatory human monocytes treated with GC in RT-PCR. By the use of ELISA, Western blot and flow cytometry analysis the differences were also confirmed on the protein level. We identified Myc, AMPK, MAPK p44/42 and 38, IL10, IL1b and other proteins related to FOXO3 to be influenced by the double stimulation.

**PSI-BR05: The role of the Kibra phosphorylation**

Jana John

PD J. Kremerskothen, Medical Clinic D

The Hippo signaling pathway regulates proliferation, growth and apoptosis. Kibra was previously described as an upstream regulator of this pathway. Its role in the Hippo pathway is primarily explained by its WW domains. These interact with other known Hippo regulators. In addition, mutations at the kibra T971 motif have been shown to induce growth defects. The negative effect of the kibra T971 dephosphorylation on size was confirmed at the organ and cellular level under endogenous conditions in this work. In addition, the functionality of WW domains was shown to be required for the negative effect on the size of the kibra T971 dephosphorylation. Interestingly, neither immunofluorescence stainings nor Western blot quantification showed enhanced Hippo activity upon kibra T971 dephosphorylation. Thus, activation of the Hippo pathway is not the underlying mechanism of size reduction. As the kinase responsible for the kibra T971 phosphorylation was not yet confirmed, the identification of the underlying mechanism was approached by clarification of the kinase. In this work, CDK4 was identified as an interaction partner of KIBRA in co-immunoprecipitation. However, it was not confirmed that this interaction is mediated through the kibra T971 motif.

**PSII-BR01**: **SYK tyrosine kinase - a key regulator of LPS-induced immune tolerance**

Jonas Wolf

Institut für Immunologie, Münster

The concept of innate immune memory specifies the functional reprogramming of immune cells, which results in an altered immune response towards a subsequent second stimulus. Tolerance of phagocytes describes an impaired response to a secondary challenge as endotoxins and is not only a well-known phenomenon in systemic infections but also in sterile inflammation. This hypo-responsiveness of phagocytes is triggered by prolonged stimulation of e.g. Toll-like receptor 4 (TLR4) with low doses of pathogenic or endogenous stimuli.

The aim of the present study was to determine the impact of non-receptor tyrosine kinase SYK for the induction of hypo-responsiveness in phagocytes. SYK is a known downstream kinase of TLR-4 and plays a pivotal role in mediating cytokine release.

Microbial tolerance was induced *in vitro* by stimulation of human monocytes with low doses of LPS for 24 h. To investigate the influence of SYK on tolerance induction, the expression and activation of SYK and its downstream target STAT1 was analyzed by qRT-PCR, western blotting and ELISA. Moreover, pharmacological inhibition of SYK and STAT1 was used to confirm their effects on the inflammatory response during tolerance.

Our analyses revealed that TLR4-dependent tolerance induction leads to a down-regulation of miRNA4800-3p which normally suppresses SYK expression. Therefore, SYK expression and its phosphorylation-dependent activation were found to be increased in tolerized cells. This was accompanied by an increased activation of STAT1. On the contrary, SYK inhibition led to an attenuated phosphorylation of STAT-1 and diminished the state of hypo-responsiveness in tolerized cells. Additionally, STAT-1 inhibition equally reversed the tolerance process.

To conclude, SYK seems to function as a novel regulator of immune tolerance by regulating the activity of STAT1 in human monocytes. This could be of functional relevance for the development of novel treatments of immune paralysis in diverse inflammatory conditions.

**PSII-BR02**: **Influence of oncogene and non-coding RNA inactivation on lung cancer metastasis**

Najeeb Shammaa

Prof. Dr. Wolfgang Berdel, Medizinische Klinik A

Lung cancer is the second most common cancer in the world. We work on a therapy method that use the small-interfering-RNA to inhibit certain genes in the tumor cells that are coding for proteins which are important for the growth and metastasis of the tumor cells. Our method is based on shut down of the proteins which are coding for example for the cMYC, KRAS, MALAT1 or NOP10 genes. To deliver the siRNA to the tumor cells we must bind the siRNA on antibodies such as anti epidermal growth factor receptor (Erbitux) or anti insulin-liked growth factor 1 receptor (Teprotumumab). to achieve that we must bind the antibodies on sulfo-SMCC-Sulfat which allow to complicate the antibodies with the siRNA.

Since the antibodies bind specific on certain receptor, we can reach a double specificity. On the first hand the antibodies will bind on the tumor cells because the receptors are overexpressed on the surface of these cells. On the other hand, the small-interfering-RNA will inhibit certain genes in these cells.

After the antibodies complex reach the cell and bind on the receptor, the hole receptor with the antibody complex will internalize to the early endosome and the to the late endosome. The acidic environment in the late endosome leads to decouple the siRNA from the hole complex and after that to liberate the siRNA in the cytoplasm. Now the siRNA can build the RISC-complex which will destroy the complementary mRNA

**PSII-BR03**: **Characterization of *Staphylococcus aureus* isolates with the same clonal background but different resistance pattern and mechanism**

Antonia Roch

Institut für Mikrobiologie, Prof. Dr. Barbara Kahl

Cystic fibrosis (CF) is one of the most common hereditary diseases. A mutation of the CFTR-channel leads to impaired muco-ciliary clearance, overproduction of mucus, especially of the respiratory system, which causes chronic recurrent bacterial infections leading to decreased pulmonary function. One of the most prevalent pathogens isolated from the airways of CF patients is *Staphylococcus aureus*. Recurrent lung infections and long-term persistence require antibiotic treatment as part of the therapy. Within this project, 20 isolates of 5 patients, which were collected during the "diversity study", were investigated. This prospective one-year study focused on the diversity of *S. aureus* in the airways of CF-patients using molecular (*spa*-typing) and phenotypical characterization of isolates and their antibiotic susceptibility. All 20 isolates were sequenced by whole genome sequencing (WGS) to determine clonal relationship and presence of resistance genes. In this work the fitness of clonal isolates with different resistance types was analyzed using competition experiments. Therefore, antibiotic-sensitive and resistant isolates were cultured together and additionally exposed to respective antibiotics for 24 hours to find out which isolate outcompetes the other. At first, the minimum inhibitory concentration against penicillin G, gentamicin and levofloxacin was determined by gradient diffusion antibiotic susceptibility testing (Etest). Clonal isolates with different resistotypes were serially diluted and plated on blood agar plates followed by bacterial counting of colony-forming units. The competition experiment showed differences in survival and fitness depending on the isolates' resistance pattern.

**PSII-BR04: „Inhibiting PI3K-AKT-mTOR signaling in Multiple Myeloma-associated mesenchymal stem cells impedes the proliferation of Multiple Myeloma cells“**

Luca Heinemann

Medical Department A

Multiple Myeloma (MM) is a relapsing hematological cancer based on the clonal expansion of a plasma cell clone in the bone marrow. Mesenchymal stem cells (MSC) represent a crucial part of the bone marrow niche and thus in the tumor microenvironment of MM. Previous studies described that MSC in MM are altered to promote the proliferation and survival of MM-cells which is mediated by cytokine release and direct cell contact. However, the molecular changes in these MM-associated MSC are not well understood. For this purpose, we carried out a transcriptome analysis to detect differentially expressed genes between MSC from MM-patients with active disease (MM-Act-MSC) and MSC from patients with other (non-) malignant diseases (CTR-MSC) and further examined MSC from MM-patients in remission.

We report that MSC from MM-patients with active disease show a distinct gene expression profile in which the PI3K-AKT-mTOR Hallmark gene set was positively enriched. As well, we detected increased levels of key proteins of the PI3K-AKT-mTOR signaling pathway in MM-Act-MSC as compared to CTR-MSC. Pictilisib, a pan-PI3K-inhibitor which can impede the proliferation of a subset of MM-cells, selectively impaired the proliferation of MM-Act-MSC meanwhile MM-Rm- and CTR-MSC were only marginally affected. Further, Pictilisib abrogated the MM-promoting properties of MM-Act-MSC.

Our data provide evidence that PI3K-AKT-mTOR signaling could be used as an additional target in the treatment of MM by not only targeting MM-cells directly, but also impeding the MM-promoting function of MSC from patients with active MM.

**PSIII-BR01**: **Characterization of the peripheral immune signature and functions of patients with relapsing-remitting multiple sclerosis treated with the immune reconstitution therapy Cladribine**

Valerie Teschner

Prof. Dr. Luisa Klotz, Klinik für Neurologie mit Institut für Translationale Neurologie

Cladribine (2-chloro-2′-deoxyadenosine) Tablets (MAVENCLAD®; Merck Serono Europe Ltd) are an approved therapeutic option in Europe since August 2017 for treatment of adults with highly active relapsing-remitting multiple sclerosis (RRMS). Cladribine therapy leads after targeted and sustained reduction of peripheral lymphocyte counts to a reconstitution of the immune regulatory network.

In this study, we characterized the depletion and reconstitution dynamics of T and B cell subsets after six and twelve months upon Cladribine therapy through multiparameter flow cytometry of 22 patients. Additionally, we examined the immune cell metabolism of CD4+ and CD8+ T cells of 23 baseline samples and 24 samples at different time points of therapy. Furthermore, we collected samples for RNA sequencing of CD4+, CD8+ and CD19+ cells of 11 patients before and after six and twelve months of therapy.

The results of the multiparameter flow cytometry indicate that Cladribine treatment lead to a significant decrease in the proportion and cell counts of memory B cells ($p = <0{,}0001$), whereas frequencies of naïve B cells were significantly increased twelve months after therapy ($p = <0{,}0001$). In comparison to the B cell compartment, we observed only minor changes in the T cell compartment. Here, we identified a significant elevation of CD4+ recent thymic emigrant (RTE) frequencies after twelve months of treatment ($p = <0{,}0001$). Moreover, we observed only a slight tendency towards lower proportions of CD4+ and CD8+ effector memory T cells during Cladribine therapy ($p_{CD4+} = 0{,}5125$, $p_{CD8+} = 0{,}2073$). Furthermore, we observed a significant decrease in the number of CD8+ T cells after 12 months of therapy ($p = 0{,}0254$). In addition, we investigated the metabolic capacity of T cells using the Seahorse/Agilent technique. Here, CD4+ T cells from patients with RRMS showed a higher oxidative phosphorylation and glycolytic function 1-12 months after Cladribine therapy. This effect was even more pronounced in CD8+ T cells after 12 months of Cladribine treatment.

Overall, our data provide evidence that Cladribine therapy leads to a decrease in differentiated lymphocytes of patients with RRMS, with more pronounced effects on the B cell compartment. In addition, our data suggest that cells exhibit a more metabolically active phenotype after therapy which may be due to immune rejuvenation.

**PSIII-BR02**: **Scrutinizing the human *TEX* genes in the context of human male infertility**

Marie Helene Sieper

Prof. Dr. Frank Tüttelmann, Institut für Reproduktionsgenetik

Infertility affects around 7% of all men worldwide. The most svere male infertility phenotype, azoospermia, is assumed to be regularly of genetic origin, with constantly emerging genes in the context of male infertility. Variants in *TEX11, TEX14,* and *TEX15* are well-established causes of azoospermia and *TEX13B*, *TEX13C,* and *TEX39B* have also been described in the context of azoospermia. However, there are 47 human genes, called "*TEX*" (testis expressed) genes, which have not been analysed systematically so far in infertile men. We screened the exome sequencing (ES) data of 1,305 men from our MERGE study for rare (minor allele frequency, MAF <1% in gnomAD database), non-synonymous variants in the coding or splice regions in accordance to the predicted mode of inheritance in all human *TEX* genes. ES data of a cohort of >5,700 proven fathers served as control cohort. Next, we generated *Drosophila melanogaster* knockdown models of 10 found *TEX* gene fly orthologues using the GAL4/-UAS system to assess the relevance of the orthologues of these genes for fertility. Our approach revealed possibly causal variants in 10 out of 47 human *TEX* genes. Hemizygous loss-of-function variants in *TEX13B*, *TEX13C* and *TEX39A* variants were detected in infertile men as well as in fertile controls. A hemizygous inframe insertion in *TEX39B* was found in two infertile men but not in controls. Knockdowns of the orthologues of *TEX2, 9, 10, 13, 27, 28, 30, 42, 261* and *292* in *Drosophila* resulted in normal reproductive phenotypes. Based on our findings, we refute previous findings that pathogenic variants in *TEX13B, TEX13C* and *TEX39A* are monogenic causes for male infertility. In contrast, we significantly strengthen evidence for *TEX39B* as a candidate gene for male infertility.

**PSIII-BR03**: ***In vivo*** **imaging of tumor-associated immune cell dynamics using time-lapse MRI**

Ina Fredrich

Tumor progression and prognosis are highly dependent on local tumor cell invasion and metastasis. One important mechanism for tumor growth is the recruitment of various immune cells to the tumor to build the tumor microenvironment (TME). Especially high numbers of tumor-associated macrophages (TAMs) are known to result in poor prognosis, but mechanisms of recruitment are still not well understood.
The project aimed to answer if and how the pro-tumoral activity of the TME alternates monocyte dynamics within the systemic circulation as a potential mechanism of cell recruitment to its TME. Therefore, time-lapse MRI of the brain vasculature as surrogate marker for systemically circulating monocytes in a syngeneic mouse model (balb/c) of breast cancer was performed and immune cell dynamics during tumor growth depending on the different malignant potential of the implanted tumor cells (highly metastatic 4T1; non-metastatic 67NR) were analyzed. Following time-lapse MRI, blood samples of mice were collected, and tumors were harvested and snap-frozen in a cryopreservation medium for *ex vivo* immunohistology analysis.
Comparison of the alteration of monocyte dynamics with respect to the underlying tumor of a low or high metastatic potential will provide further understanding of how high malignant tumors promote their progress via systemic immune cell manipulation and if time-lapse MRI enables to noninvasively assess tumor-associated alteration in the dynamics of monocytes within the systemic circulation *in vivo*.

**PSIII-BR04**: **Genetic polymorphisms in the promoter of the FSHB gene and their impact on transcription factor binding**

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Prof. Jörg Gromoll, CeRA

Follicle stimulating hormone (FSH) plays a major role for male fertility by targeting Sertoli cells in the testes, which function as “nursing cells” and are essential for spermatogenesis. Its beta-subunit (FSHB) defines the amount of synthesized hormone, thus, FSHB transcription is the limiting step and determines serum levels of FSH. SNPs (Single-nucleotide-polymorphisms), a variation of a single base pair in the DNA, are the most common type of genetic variations. One particular SNP (rs 10835638, G>T), located in the FSHB promoter -211 base pairs upstream of the gene, leads to a reduction of FSH serum levels and infertility in males with a T allele. Previous studies showed that the -211 SNP is located within one of the binding sites of the transcription factor LHX3, which is known to influence FSHB transcription. We modified the FSHB promoter by in-vitro mutagenesis in which we delete or substitute certain parts of the DNA. Functional analyses were performed in the 3 different cell lines Hek293, CHO and the pituitary cell line LßT2 using a luciferase assay. We then identified other LHX3 binding sites in the FSHB promoter via bioinformatical analysis and tested their importance for FSHB biosynthesis because we hypothesized that the -211 SNP rs10835638 is potential cross-linked with other genomic that exert the observed effect. In a second round of luciferase assays we individually deleted the 6 binding sites (A-F) that we found in our bioinformatical analysis and identified those ones with a significant effect on FSHB transcription.

**PSIII-BR05**: **„The action of follicular fluid on Slo3 channels from human sperm“**

Clara Eisenhardt

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During their journey through the female genital tract, sperm encounter changes in pH, electrolyte concentrations and viscosity of the environment. Sperm sense these changes using a repertoire of sperm-specific ion channels, which translate changes in the chemical microenvironment into changes of the intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) and swimming behaviour. Still it is not clear how exactly the variations of the environment affect the ion channel activity and how that translates into changes in sperm motility.

Slo3 is a sperm-specific potassium channel that is controlled by the intracellular calcium and pH. It controls the membrane potential and, thereby, multiple voltage-dependent ion channels and transporters. In this project, we investigate the action of follicular fluid, which enters the oviduct upon ovulation, on Slo3. We use patch-clamp recordings to study the activity of human Slo3 channels. Follicular fluid contains steroid hormones, as progesterone, that inhibit Slo3 channels, but the action of the different steroids is not sufficient to explain its action on Slo3 channels. Beside steroids it contains mainly growth factors, sugars and proteins. Here we could show that follicular fluid contains large amounts of albumin, and identified this protein as the missing active component.

**PSIII-BR06**: **“Detection of stress signals in *Escherichia coli* using a fluorescent reporter gene-based system: Primary targets of different phytopharmaceuticals”**

David Tutschner

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Over the last decades, antibiotic resistance has become an increasing problem in the treatment of bacterial infections. The number of antibiotic-resistant infections in hospitals increase, and many antibiotics show a decreasing effect against resistant bacteria. In addition, only a few new antibiotics were introduced into the market over the last decades. The WHO has called out antimicrobial resistance in general as one of the biggest 10 threats to humanity, and declared a post-antibiotic era, where antibiotics will lose their therapeutic effect against many bacterial infections.

The increasing number of resistant isolates in infections and the lack of new antibiotics makes it crucial to find new therapeutic options. One approach is the so-called combination therapy, where you combine antibiotics with non-antibiotic drugs that cause stress in bacteria. Due to the non-antibiotic drugs, it is possible to sensitise the bacteria for the antibiotics and as a result restore their therapeutic effect.

Detecting possible drugs for combination therapy can be achieved by screening known drugs for their stress induction and growth inhibition of bacteria. We use a reporter system in *Escherichia coli* K-12 to measure specific stress pathways like pH, envelope, and oxidative stress. When a certain stress is induced, the reporter system produces the fluorescent protein YFP which can be detected over time due to kinetic measurement. The set of test substances included several phytopharmaceuticals that are used in urinary tract infections.