

21st International Symposium

hosted by students of the
Graduate School of Life Sciences

EUREKA! 2026

October 7-8, 2026
at Rudolf Virchow Center



ABSTRACT BOOK

21st International Symposium

hosted by students of the
Graduate School of Life Sciences



EUREKA! 2026 Program

October 7th

09:00 WELCOME ADDRESS

09:30 **Lenka Bešše**

The Plasticity of Proteostasis — How Myeloma Cells Rewire to Survive Proteasome Inhibition

10:00 **Christina Ottemeier**

Targeting the MYCN-Aurora A-CDK 12 axis in Neuroblastoma

10:15 **Shaliya Hashardeen**

One enzyme, two fates: dual specificity of UBA6 in lung cancer

10:30 **Annika Köhler**

Evaluation of a VR Simulator to Train Gastric Bleeding Treatment with Argon Plasma Coagulation and Injection

10:45 COFFEE BREAK

11:15 **Anna Stöckl**

More than just a pretty flower: how visual patterns guide insect – plant interactions

11:45 **Johanna Ott**

Beyond Bombus terrestris: Wild bumblebee species are more sensitive to commonly used pesticide than model species

12:00 **Séamus Holden**

How do you build a wall? Using super-resolution microscopy to understand the biophysics of bacterial cell wall remodelling

12:30 LUNCH BREAK

13:30 **POSTER SESSION**

15:00 COFFEE BREAK

15:30 **Marisa Karow**

Decoding the molecular and cellular drivers of long-range neuronal connections

16:00 **Hanwei Liu**

A Containerized Toolbox for Directed Connectivity in Multimodal Neuroscience

16:15 **Felix Meissner**

Rewiring of Inflammatory Signaling at Proteome Scale

16:45 CLOSING REMARKS

October 8th

08:45 OPENING REMARKS

09:00 **Jost Enninga**

Deciphering the molecular basis how bacterial pathogens find their preferred intracellular niche(s) - The power of correlative imaging workflows and single cell tools

09:30 **Anagha Rajeevalochana**

Soluble chemokine gradients direct splenic type 1 dendritic cell migration along perivascular tracks

09:45 **Kumari Monika**

SnRK1 dependent transcription factors controlling Arabidopsis seedling germination and establishment

10:00 **Aybaran Olca Kebabci**

Actin conformation dynamics precede force generation in cytotoxic T lymphocytes

10:15 COFFEE BREAK

10:45 **CAREER SESSION**

Judith Gabel

Science Communication Lab - Deutsches Museum München

Dennis Eckmeier

Science Journalist

Barbara Hissa

Managing Editor of Beilstein Journal of Nanotechnology

Tobias Kerrinnes

Scientific Infrastructure HIRI

12:30 LUNCH BREAK

13:30 **POSTER SESSION**

15:00 COFFEE BREAK

15:30 **Lucas Massier**

More Than Fat: The Hidden Complexity of Adipose Tissue

16:00 **Hildegard Büning**

Tailoring Adeno-Associated Virus (AAV) vectors to improve efficacy of in vivo gene therapy

16:30 **AWARD CEREMONY**

16:45 CLOSING REMARKS



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KEYNOTE SPEAKER

7.Oct, 9:30-10:00



Lenka Bešše

Masaryk University, Brno, Czech Republic

Dr. Lenka Bešše is a cancer biologist and Junior Group Leader at the Department of Biology, Faculty of Medicine, Masaryk University, Brno, Czechia. She earned her PhD in Oncology from Masaryk University in 2014 before undertaking postdoctoral research at the Cantonal Hospital St. Gallen, Switzerland. From 2017 to 2023, she served as Head of the Laboratory of Experimental Oncology at the Cantonal Hospital St. Gallen, where she established an independent research program investigating mechanisms of cancer therapy resistance. Her current research focuses on proteostasis, protein homeostasis, and tumor adaptation to chemotherapy and immunotherapy.

The Plasticity of Proteostasis — How Myeloma Cells Rewire to Survive Proteasome Inhibition

Multiple myeloma is a plasma cell malignancy in which proteasome inhibitors have dramatically improved patient survival. Yet despite remarkable initial responses, resistance inevitably develops. What enables cancer cells to survive a therapy that so profoundly disrupts protein homeostasis? In this talk, I will discuss how studies of proteasome inhibitor resistance have revealed the striking plasticity of the proteostasis network. Rather than relying on a single resistance mechanism, myeloma cells coordinate a rewiring of proteasome function, energy metabolism, redox balance, and protein folding to restore cellular homeostasis under sustained therapeutic stress. Understanding these adaptive processes opens new avenues to overcome drug resistance, while also revealing broader principles of how cells respond to proteotoxic stress — with implications for cellular adaptation and evolution under selective pressure beyond multiple myeloma.

7.Oct, 11:15-11:45



Anna Stöckl

University Konstanz, Germany

Anna Stöckl has been Junior Professor of Neuroethology at Konstanz University since 2023. Her research investigates neural processing in the insect visual system that underlies their natural behaviour. Using pollinating insects as model systems, her group combines neurophysiology, behavioural analysis, neuroanatomy, environmental imaging, and modelling. Her research focuses on vision under changing light conditions, insect-flower interactions and sensorimotor control of movement. She studied Biology at Heidelberg University and Neuroscience at the LMU Munich, earned her PhD at Lund University in Sweden, and held research positions at Aalto University in Finland and the University of Würzburg before establishing her own group.

More than just a pretty flower: how visual patterns guide insect – plant interactions

We have all experienced the fascinating range of colourful patterns that flowers display – though while merely pleasing to us, they can be of great importance to animals that visit flowers for their daily food supply. Flower patterns are thought to lead insect pollinators to a plant's nectary, expressed in the term 'nectar guides'. I will present recent work on how flower patterns guide the flower interactions of two insect pollinators, the buff-tailed bumblebee (*Bombus terrestris*), and the hummingbird hawkmoths (*Macroglossum stellatarum*). These two species have very different flower-interaction strategies, as bumblebees land on the flowers and make their way to the nectary on foot, while hawkmoths hover in front of them and search for the nectary with their long proboscis. I will provide insights how this guidance function of flower patterns might have shaped innate preferences of pollinators for flower patterns, and how these laboratory pattern preferences translate to flowers visited by these insects in nature, based on citizen science observation data. The talk will thus highlight our work on sensory-motor control, cognitive strategies, and co-evolutionary hypotheses concerning the role of flower patterns in insect-plant interactions.

8.Oct, 12:00-12:30



Séamus Holden

University of Warwick, UK

My lab uses advanced light microscopy to study the biophysics of bacterial growth and division. We study how proteins function as nanoscale machines that build complex microscale structures. We particularly focus on proteins that build and remodel the bacterial cell wall, which is one of the most important targets for antibiotics. To achieve this we use super-resolution microscopy to follow the activity of individual cell wall synthesis proteins at nanoscale resolution inside live bacteria.

2024: Promoted to Professor, University of Warwick. 2022: Moved to University of Warwick as Associate Professor (Reader). 2020: Awarded British Biophysical Society Louise Johnson Early Career Award. 2018: Promoted to Principal Research Associate, Newcastle University. 2017: Awarded Wellcome Trust & Royal Society Sir Henry Dale Fellowship. 2015: Moved to Newcastle University to start independent research career. 2012: Awarded Marie Curie Intra-European Fellowship. 2011-2015: Post-doctoral Researcher in laboratory of Professor Suliana Manley, EPFL, Switzerland. 2007-2011: DPhil in Biological Physics in laboratory of Professor Achilles Kapanidis, University of Oxford. 2003-2007: MPhys in Physics, University of Oxford.

How do you build a wall? Using super-resolution microscopy to understand the biophysics of bacterial cell wall remodelling

Almost all bacteria are surrounded by a mesh-like peptidoglycan cell wall essential for their survival. Defects in cell wall structure cause bacteria to burst and die due to the cell's high internal osmotic pressure. Many rod-shaped bacteria, including major antibiotic resistant pathogens, elongate by adding new material to the sides of their cell wall. In these organisms, an essential multi-protein synthesis complex called the elongasome inserts glycan strands around the circumference of the cell, elongating and reinforcing the cell wall and giving cells their rod shape. How cells regulate the length of circumferential glycan strands and the effect of circumferential glycan strand length on cell size and shape is largely unknown.

We used single particle tracking (SPT) to measure the length of individual synthesis events (processivity) of the *Bacillus subtilis* elongasome, which determines the initial length of new glycan strands [1]. We found that elongasomes are highly processive, and that *B. subtilis* elongasome dynamics and processivity are determined by a balance between processive synthesis and molecular

motor tug-of-war, where multiple synthases pull individual MreB filaments in opposite directions. We also found evidence that elongasome processivity and tug-of-war regulate *B. subtilis* cell size and shape.

In unpublished work, we have been further investigating the molecular mechanisms of elongasome tug-of-war using a combination of bacterial genetics, SPT and super-resolution microscopy. We have also applied MINFLUX SPT to this problem, giving measurements of elongasome dynamics with at least an order of magnitude improvement in spatiotemporal resolution than obtained previously by conventional single . I will present recent progress in this area.

[1] S. Middlemiss, M. Blanden, D.M. Roberts, A. McMahon, J. Grimshaw, J.M. Edwards, Z. Sun, K.D. Whitley, T. Blu, H. Strahl, S. Holden, Molecular motor tug-of-war regulates elongasome cell wall synthesis dynamics in *Bacillus subtilis*, *Nat. Commun.* 15 (2024) 5411. <https://doi.org/10.1038/s41467-024-49785-x>.

7.Oct, 15:30-16:00



Marisa Karow

FAU Erlangen-Nürnberg, Germany

Marisa Karow has been Associate Professor at the Institute of Biochemistry at Friedrich-Alexander University Erlangen-Nürnberg since 2019. Her research investigates the mechanisms that determine neural cell identity and how these can be harnessed to understand human brain development and disease. Using human stem cells and brain organoids as model systems, her work combines cellular reprogramming with molecular and cellular analyses. Her research focuses on the conversion of non-neuronal cells into neurons, neural cell differentiation, and neurodevelopmental disorders. She studied Biology and earned her PhD at LMU Munich, and held postdoctoral research positions at LMU Munich and Stanford University. She subsequently led a research group at LMU Munich before taking up her professorship in Erlangen, where she received tenure in 2025.

Decoding the molecular and cellular drivers of long-range neuronal connections

Successful corticogenesis depends on tightly coordinated molecular and cellular programs that guide the formation of neuronal connections. However, the mechanisms that enable neurons to extend and guide axons over long distances remain incompletely understood. Here, we investigate the molecular and cellular principles underlying long-range connections development using human brain organoids. Modelling three neurodevelopmental disorders with long-range connections defects: Coffin-Siris Syndrome (SOX11 heterozygous), Pitt-Hopkins Syndrome (TCF4 heterozygous) and Optiz/BBB-G Syndrome (MID1 knock-out), we identify convergent cellular and molecular phenotypes in neurons uncovering central mechanisms governing neurite outgrowth and guidance. We find that neurons consistently exhibit reduced neurite growth speed and impaired directional persistence, with deficits becoming pronounced over longer distances. Single-cell transcriptomics reveals convergence at more integrative levels, highlighting dysregulation of energy metabolism, polyunsaturated fatty acid (PUFA) pathways, and cell–extracellular matrix (ECM) interactions as core features associated with impaired formation of long-range neuronal connections. Combining in silico modeling and live imaging, we demonstrate that modulation of growth cone geometry—specifically, the opening angle—is sufficient to alter neurite trajectory and recapitulate key aspects of impaired neurite growth. Functional manipulation of PUFA metabolism show direct impact in growth cone morphodynamics, linking PUFA metabolism directly to the physical behavior of extending neurites. Together, these findings identify growth cone dynamics, neuron–ECM interactions, and lipid metabolism as interacting drivers of long-range neuronal connection formation. This work provides a framework for understanding how molecular and cellular processes integrate to shape large-scale neuronal wiring.

7.Oct, 16:15-16:45



Felix Meissner

University of Bonn, Germany

Felix Meissner studied biochemistry at the Free University of Berlin and the Scripps Institute in San Diego, USA. He obtained his doctorate at the Max Planck Institute for Infection Biology in Berlin. His postdoctoral work then took him to the Max Planck Institute of Biochemistry in Martinsried and then to the University of California in San Francisco. He then returned to Munich as an independent Research Group Leader for "Experimental System Immunology" at the same Institute. Since 2021 he is heading the Department of "Systems Immunology and Proteomics" at the Institute of Innate Immunity in Bonn. He has co-founded Odyssey Therapeutics, which translates discoveries into novel therapeutics.

Rewiring of Inflammatory Signaling at Proteome Scale

Sterile inflammatory diseases have become a major health and socioeconomic burden, yet the molecular mechanisms that govern the initiation and propagation of inflammation remain incompletely understood. Mass spectrometry-based proteomics has emerged as a powerful discovery tool to systematically investigate signaling circuits within and between cells. I will present promising biochemical, proteomic, computational, and immunological strategies to dissect complex inflammatory reactions and identify proteins and proteoforms with previously unexplored bioactivities on cellular and organism level.

Focusing on extracellular proteins, our analyses uncover a previously hidden layer of cellular crosstalk driven by unannotated cytokines and proteolytic proteoforms that diversify and reshape immune signaling. Many of these variants arise from endogenous protease activity and display context- and tissue-specific usage. Deep plasma proteomics in human cohorts highlights the translational potential of proteoform-aware profiling for sensitive monitoring and stratification of inflammatory activity. Together, our work establishes a proteome-scale foundation to decode and clinically harness inflammatory signaling.

8.Oct, 9:00-9:30



Jost Enninga

Institute Pasteur, Paris, France

Jost Enninga heads the research unit « Dynamics of host-pathogen interactions » within the Department of Cell Biology and Infection at the Institut Pasteur in Paris. Jost studied biochemistry in Hannover, Germany, and then he performed his Ph.D. in the Laboratory of Cell Biology at the Rockefeller University, New York, under the guidance of Dr. Guenter Blobel and Dr. Beatriz Fontoura (2000-2004). There, he focused on how viruses subvert host cellular trafficking processes. Jost moved to the Institut Pasteur in Paris to study the early events of bacterial host cellular crosstalk with Dr. Philippe Sansonetti and Dr. Guy Tran Van Nhieu (2004-2008). Between 2008 and 2012, Jost formed an independent junior team at the Institut Pasteur to investigate the internalization of the human pathogen *Shigella* into host cells. Since 2013, he heads his own research unit at Institut Pasteur studying the intracellular niche formation of bacterial pathogens, and he has been deputy as well as acting director of the Cell Biology and Infection Department between 2014 and 2019. He has also been serving on many national and international committees, including INSERM, the CNRS, the DFG. Apart from doing science, Jost has also been involved in many training courses that focus on the integration of advanced imaging techniques for the study of microbes. For example, he has run a series of EMBO workshops in South Africa, and he has implemented a training course at the Institut Pasteur on advanced optical imaging.

Deciphering the molecular basis how bacterial pathogens find their preferred intracellular niche(s) - The power of correlative imaging workflows and single cell tools

Our research unit studies the fundamental question how intracellular pathogens manipulate host pathways to reach a specific intracellular niche. As models we use two important enteroinvasive bacteria *Shigella*, *Salmonella* as well as *Mycobacterium tuberculosis*, and we challenge non-phagocytic as well as professional phagocytic cells with these microbes. For our research, we have been emphasizing the implementation of single-cell approaches. These methods comprise the development of novel fluorescent biosensors, high-content screening, correlative light-electron microscopy (CLEM) under cryo conditions, and microfluidic-based single-cell OMICs. This enables us to (i) identify the major cellular factors involved in the underlying processes (e.g. niche formation of the studied pathogens, pathways traveled during drug delivery, and responses induced), to (ii) decrypt the mechanisms and molecular cascades induced, and to (iii) obtain in situ structural information at the angstrom level of the key molecular complexes.

8.Oct, 15:30-16:00

Lucas Massier



Helmholtz Institute, Munich / University of Leipzig, Germany

Lucas Massier is a Principal Investigator and Junior Group Leader at the Helmholtz Institute for Metabolic, Obesity and Vascular Research (HI-MAG) in Leipzig, Germany, where he leads the research group *Adipose Tissue Microarchitecture and Metabolism*. He studied Biochemistry and Molecular Cell Biology and Molecular Medicine at Friedrich Schiller University Jena before completing his PhD in Biochemistry and Experimental Endocrinology at the University Hospital Leipzig in 2020. His doctoral research investigated bacterial translocation to adipose tissue and its relationship with metabolic disease. He subsequently moved to the Karolinska Institutet in Stockholm, where his research expanded towards the cellular organization and function of human adipose tissue.

Today, his research focuses on understanding how the complex cellular architecture of adipose tissue contributes to metabolic health and disease. His group combines single-cell and spatial transcriptomics with proteomics, epigenomics, and clinical data to study adipose tissue across anatomical depots and throughout the human lifespan. A particular focus is understanding how different cell types interact within adipose tissue and how specialized depots, including fat surrounding the intestine, sense and respond to nutritional, microbial, and environmental signals. He also initiated and co-led *adiposetissue.org*, a public knowledge portal integrating molecular and clinical datasets from human adipose tissue.

More Than Fat: The Hidden Complexity of Adipose Tissue

For a long time, adipose tissue was viewed primarily as an energy store and a cushion protecting our organs. Modern single-cell and spatial technologies are changing this view. Although adipocytes occupy around 90% of the physical volume of adipose tissue, they account for only about 20% of its cells. The remaining cellular landscape consists of immune cells, vascular cells, fibroblasts, and diverse progenitor populations, all organized into a complex three-dimensional microarchitecture.

This cellular organization is not simply structural. It influences how adipose tissue responds to insulin, regulates immune responses, and enables communication between different cell types. Spatial studies have, for example, revealed distinct adipocyte populations with different sensitivities to insulin and specific interactions between stromal, immune, and vascular cells.

Importantly, there is no single “adipose tissue”. Different fat depots throughout the body have distinct cellular compositions, spatial organization, and functions. Recent work mapping multiple human depots has shown particularly striking specialization in adipose tissue surrounding the intestine and colon. These depots contain specialized adipocytes and immune cell populations and appear to be adapted to their proximity to the gut and its microbial environment.

This raises a broader question: could adipose tissue serve as an important interface between our internal organs and the environment? In particular, fat surrounding the intestine may sense nutritional and microbial signals, coordinate local immune responses, and communicate these signals to the rest of the body.

In this talk, I will explore how looking beyond adipocytes, and beyond fat as a storage organ, is revealing adipose tissue as a highly organized and dynamic regulator of human physiology.

8.Oct, 16:00-16:30



Hildegard Büning

Hannover Medical School, Germany

Hildegard Büning is Professor of Experimental Cell and Gene Therapy Infection at Hannover Medical School (Germany). She is Director of the Hannover Center for Gene and Cell Therapy and Deputy Director of the Institute of Experimental Hematology. She served as President of the European Society of Gene and Cell Therapy (www.ESGCT.eu, 2018-2022), as Member of the Board of Directors of the American Society of Gene & Cell Therapy (<https://asgct.org/>; 2021-2024) and is the Scientific Secretary of the German Society for Gene Therapy (www.dg-gt.de/; since 2014).

She studied Biology and obtained her PhD (Dr. rer. nat.) at the Ludwig-Maximilians-University Munich (Germany) in 1997. Since then, she is active in the field of adeno-associated virus (AAV) vectors with a particular focus on AAV-host interactions and vector development. Aiming to improve efficacy and safety of AAV vectors for gene therapy, she is tailoring vector tropism and immunogenicity using both rational design and library-based approaches.

Tailoring Adeno-Associated Virus (AAV) vectors to improve efficacy of *in vivo* gene therapy

AAV vectors are biological nanoparticles derived from replication-deficient adeno-associated viruses (AAV). They are currently the most widely used delivery system for *in vivo* gene therapy; maybe best highlighted by the increasing numbers of AAV vector-based *in vivo* gene therapies entering clinical routine. AAV vectors are applied for gene addition (or gene supplementation) as well as gene editing strategies. The decision of whether to apply AAV vectors locally or intravenously is dependent upon the disease and, thus, dependent upon the main target cell type. Compared to *ex vivo* gene therapy strategies, *in vivo* applications face the challenge of the high prevalence of pre-existing neutralizing antibodies in the human population due to exposure to the wild-type virus. Moreover, even when applied locally, vectors will be lost in off-target cell types due to the generally broadtropism of vectors based on naturally occurring viruses. A further challenge is the high vector dose that is applied to overcome pre- and post-entry barriers towards transduction. In response to these challenges, capsid engineering has been developed more than 25 years ago. Since then, the AAV toolbox has been greatly expanded with capsid-engineered AAV vectors clearly outperforming AAV vectors derived from naturally-occurring serotypes regarding efficacy as delivery tools for treating monogenetic disease. Moreover, due to these technical advances and gained insights into the host-vector interaction, the potential medical utility of AAV vectors is expanding to new “fields of action”. For example, capsid engineered AAV vectors are explored as vaccine platforms, to improve outcome for patients receiving implants, to improve *ex vivo* gene therapy approaches and to move *ex vivo* gene therapies into *in vivo* applications.

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STUDENT SPEAKER



7.Oct, 10:00-10:15



Christina Ottemeier

Targeting the MYCN-Aurora A-CDK 12 axis in Neuroblastoma

Christina Ottemeier (AG Büchel, Medical doctoral researcher, GSLS), Mareike Solvie PhD (AG Büchel, department of biochemistry and molecular biology- Biozentrum Würzburg), Prof. Dr. Gabriele Büchel (Department of biochemistry and molecular biology, professorship for the dynamics of cellular protein complexes, Biozentrum Würzburg)

Neuroblastoma is the most common extracranial solid tumour in children, with MYCN amplification associated with aggressive disease and poor outcome. In S phase, Aurora A forms a complex with MYCN and activates CDK12, promoting the recruitment of transcription termination complexes. The described mechanism plays an important role in protecting cancer cells from transcription-replication conflicts. Aurora A inhibition induces replication stress and compensatory ATR activation. Since single agent therapies often lead to resistance due to the high tumour heterogeneity and the activation of alternative pathways, our aim is to characterize the impact of targeting the MYCN-Aurora-A-CDK12 axis comparing single-agent treatment and combination therapies.

We investigated the effect of combinatorial Aurora A and CDK 12 inhibition on cell viability and cytotoxicity using the Aurora-A inhibitor TAS119 and the CDK12 inhibitor CT7439. We identified drug concentrations that caused minimal general cytotoxicity individually but showed additive effects on MYCN-amplified neuroblastoma cell viability. To determine whether combinatorial inhibition affects apoptosis and cell cycle progression, we performed flow cytometry analysis. BrdU/PI assays showed that combinatorial treatment caused a redistribution of cell cycle populations. This suggested a disruption of S phase progression, potentially leading to S phase arrest, along with increased cell death, reflected by an enlarged sub-G1 population. Using Annexin-V/PI assays, we detected that combinatorial treatment induces higher levels of apoptosis than single-agent-treatment. To further assess DNA damage induced by combinatorial treatment, we will analyse different DNA damage markers using immunoblotting. To characterize the molecular consequences of the combinatorial treatment, we will assess the accumulation of RNA-DNA hybrid structures and perform proximity ligation assays investigating transcription regulation. In parallel, we will assess how these interventions affect phosphorylation of the RNA polymerase II C-terminal domain, a key regulator for productive transcriptional elongation and transcription termination.

Although previous studies have demonstrated the effects of the individual inhibitors, this study aims to establish whether combinatorial targeting Aurora A, CDK12 and ATR provides a promising therapeutic strategy for aggressive, MYCN-amplified neuroblastoma.

7.Oct, 10:15-10:30



Shaliya Hashardeen

One enzyme, two fates: Dual specificity of UBA6 in lung cancer

Shaliya Hashardeen (1), Markus Diefenbacher (2-4), Hermann Schindelin (1) (1) Institute of Structural Biology, Rudolf Virchow Center for Integrative and Translational Bioimaging, Julius-Maximilians-Universität Würzburg, Germany E-mail:shaliya.puthanveedu-hashardeen@uni-wuerzburg.de (2) Helmholtz Center Munich and German Center for Lung Research (DZL) Munich, Germany (3) Ludwig-Maximilians-Universität, Munich, Germany (4) German Center for Translational Cancer Research (DKTK), Munich, Germany

UBA6 is a unique E1 activating enzyme with dual specificity for ubiquitin and the ubiquitin-like modifier FAT10, yet whether and how this bifunctionality contributes to cancer remains poorly understood. Here, we investigate UBA6-mediated ubiquitylation and FATylation as functional dependencies in non-small-cell lung carcinoma. Using endogenous depletion of UBA6, and FAT10[1], we demonstrate that a representative panel of lung cancer cell lines show pronounced dependence on this pathway for cellular fitness, positioning UBA6 as a regulator of tumor maintenance. To delineate the relative contribution of ubiquitylation versus FATylation, we established a genetic complementation system where the endogenous UBA6 locus is disrupted using intron-specific sgRNAs and replaced it with exogenous UBA6 variants selectively defective in either ubiquitin or FAT10 activation [2]. Comparative analysis revealed that UBA6-mediated ubiquitylation and FATylation contribute to different biological processes in tumor. In parallel, we identified candidate oncogenic substrates modified by FAT10, linking FATylation to altered protein stability and tumor-relevant signaling pathways. Together, our findings support UBA6 as a critical node, integrating ubiquitin and FAT10 pathways in lung cancer and reveal exploitable vulnerabilities arising from its dual enzymatic activity.

References

- [1] Aichem, A. et al. Nat Commun. 9. (2018)
- [2] Truongvan, N. et al. Nat Commun 13. (2022)

7.Oct, 10:30-16:45



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Annika Köhler

Evaluation of a VR Simulator to Train Gastric Bleeding Treatment with Argon Plasma Coagulation and Injection

A. Köhler 1, I.A. Syed 1, J.S. Theile 1, S. Dimitriadis 2,3, I. Kafetzis 1, J. Troya 1,4, R. Pryss 5,6, B.M. Walter 7,8, A. Meining 1,4, A. Hann 1 1 Interventional and Experimental Endoscopy (InExEn), Department of Internal Medicine II, University Hospital Würzburg, Würzburg, Germany 2 School of Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece 3 Gastroenterology Private Practice, Thessaloniki, Greece 4 Bavarian Center for Cancer Research (BZKF), Würzburg, Germany 5 Institute of Clinical Epidemiology and Biometry, University of Würzburg, Würzburg, Germany 6 Institute of Medical Data Science, University Hospital of Würzburg, Würzburg, Germany 7 Department of Internal Medicine I, University Hospital Ulm, Ulm, Germany 8 Endoscopy Research Group, University Hospital Ulm, Ulm, Germany

Introduction: Gastric bleeding has potentially severe consequences for the patient. While training its treatment is therefore crucial, opportunities for hands-on practice are limited. A virtual reality (VR) application provides a risk-free and accessible solution. This study evaluates a VR training simulator for gastric epinephrine injection and argon plasma coagulation (APC).

Methods: A VR training simulator was developed to virtually train the treatment of gastrointestinal emergencies by operating a conventional endoscope tracked by sensors. In a user study, 20 participants completed task one, treating an angiodysplasia via APC, and task two, treating a bleeding angiodysplasia via submucosal injection followed by APC. Participants assessed the simulator based on workload (raw NASA-TLX), usability (System Usability Scale, SUS), cybersickness, immersion, face and content validity (5-point Likert scale: 1 strongly disagree, 5 strongly agree), and gave free-text feedback. The simulator further provided measurements on task duration, percentage of treated angiodysplasia area, and successful hemostasis. Reported were means (M) and medians (Mdn), and the results of subgroup analyses.

Results: Participants rated the VR training simulator as especially useful for novices, prior to first patient intervention, and favored its adoption into endoscopy training (all Mdn = 5). They further perceived the simulator as helpful for training APC and injection (all Mdn = 4). Participants additionally rated the stomach anatomy (Mdn = 5), the stomach texture, endoscope footage and movement, the application of APC, and the instruments as highly realistic (all Mdn = 4). In general, the simulator had good usability (SUS M = 73.63) and was associated with low workload (NASA-TLX M = 35.63) and almost no cybersickness (Mdn = 1). More experienced endoscopists had shorter intervention times than less experienced endoscopists for both task one (M = 50.8s vs 107.0s, $p = .008$) and task two (M = 96.2s vs 245.7s, $p = .012$). Participants further achieved higher scores for area treatment in task one than in task two (M = 66.9% vs 55.2%). Among the 20 participants, 95% achieved successful hemostatic control.

Conclusion: The results of this study suggest the VR simulator's potential as a training tool for the treatment of gastric bleeding via APC and injection therapy. Such a simulator enables risk-free practice while reducing animal-based training.

7.Oct, 11:45-12:00



Johanna Ott

Beyond *Bombus terrestris*: Wild bumblebee species are more sensitive to commonly used pesticide than model species

Johanna Ott, Abdulrahim Alkassab, Ricarda Scheiner, Ingolf Steffan-Dewenter

Agrochemicals are among the most important drivers of pollinator declines. Both lethal and sublethal effects can severely impact individuals and for social bees, colony development. A harsh decline in numbers has also been observed for bumblebees, an important group of pollinators in European landscapes.

Most ecotoxicological studies on bumblebees focus on the buff-tailed bumblebee *Bombus terrestris* due to easy access to commercial colonies and convenient colony rearing. With signs accumulating that this species is not always representative of others, a wider range of species needs to be tested to assess the impact of agrochemicals.

For this reason, we conducted the first ecotoxicological assay on two common, yet understudied bumblebee species, *Bombus pascuorum* and *Bombus sylvarum*. Following official OECD guidelines, we assessed lethal endpoints to Chlorantraniliprole, a commonly used insecticide. We further investigated whether field-realistic concentrations of this compound impacted sublethal behaviors such as feeding and locomotion.

We found that *B. pascuorum* and *B. sylvarum* are more sensitive to Chlorantraniliprole than previous records of *B. terrestris* and that field-realistic concentrations suffice to impair feeding and movement patterns. This study is the first to conduct ecotoxicological assays on those two bumblebee species and it suggests that current risk assessment procedures underestimate the impact of agrochemicals on wild bumblebee species.

7.Oct, 16:00-16:15



Hanwei Liu

A Containerized Toolbox for Directed Connectivity in Multimodal Neuroscience

Hanwei Liu, Manuel Bange, Philip Tovote, Muthuraman Muthuraman

Understanding how information propagates through brain networks requires reliable estimates of directed interactions among neural populations. However, the inferred network topology is shaped not only by the underlying neural dynamics, but also by the choice of connectivity estimator, preprocessing strategy, and software environment. These dependencies complicate comparisons across methods and limit reproducibility, particularly when signals from different recording modalities are integrated.

In this talk, we present a reproducible, containerized toolbox for estimating and benchmarking directed connectivity in multivariate neural time series. The workflow combines BIDS-compatible data handling, modality-aware preprocessing, version-pinned computational environments, automated pipeline execution, standardized source–target conventions, and statistical validation. It integrates complementary estimator families, including conditional Granger causality, directed transfer function, partial directed coherence, phase-slope index, and transfer entropy, while retaining undirected association measures as comparative controls.

We benchmark these methods on linear and nonlinear simulated networks with known directed topology and quantify network recovery using complementary performance metrics. The analyses show that inferred topology and estimator performance vary across network dynamics and methodological assumptions, emphasizing the value of comparing convergent and divergent evidence across measures rather than interpreting a single connectivity matrix in isolation. We further apply the workflow to simultaneous MEG and subthalamic local field potential recordings from people with Parkinson’s disease to investigate beta-band interactions between motor cortex and the subthalamic nucleus across medication and motor conditions. By connecting controlled network reconstruction with real-world multimodal analysis, this framework provides a practical route towards reproducible and interpretable studies of directed information processing in brain networks.

8.Oct, 09:30-09:45



Anagha Rajeevalochana

Soluble chemokine gradients direct splenic type 1 dendritic cell migration along perivascular tracks

Anagha Rajeevalochana¹, Augusto Velozo Gonçalves^{1,10}, Reuben Jacob Labios^{1,10}, Deeksha Seetharama¹, Xiaoli Yang¹, Nagham Alouche², Serena Melgrati³, Johanna Merlin⁴, Lisa Marschhäuser⁵, Tsuneyasu Kaisho^{6,7}, Michael Sixt⁸, Yinming Liang⁹, Marcus Thelen³, Georg Gasteiger¹, Sanjiv Luther², Milas Ugur¹, Marc Bajénoff⁴ and Wolfgang Kastenmüller¹

Migration of dendritic cells (DCs) is essential for their ability to relay antigenic information from peripheral tissues and instruct antigen-specific T cells within lymphoid organs. DC migration is driven by CCR7-dependent haptotaxis along immobilized CCL21 gradients toward lymphatic vessels, enabling transport to draining lymph nodes. How DC migration is organized within the spleen, an organ specialized in blood surveillance, has remained unclear. Here, using high-resolution confocal microscopy and functional perturbations, we show that splenic cDC1 migrate from the red pulp to the white pulp by following perivascular tracks surrounding arterioles – the same pathways used by lymphocytes entering from the bloodstream. Strikingly, this migration is guided not by immobilized chemokine cues but by long-range gradients of soluble CCL19 and cleaved CCL21. Although these chemokines are produced within the white pulp, they are distributed along perivascular spaces toward the venous sinuses of the red pulp establishing a directional chemotactic gradient. Thus, splenic DC migration operates through a paradigm fundamentally distinct from lymphatic DC trafficking, relying on chemotaxis rather than haptotaxis. Together, our findings define the anatomical pathways and molecular mechanisms underlying DC migration within the spleen and reveal how vascular organization shapes immune cell positioning.

8.Oct, 09:45-10:00



Kumari Monika

SnRK1 dependent transcription factors controlling Arabidopsis seedling germination and establishment

Kumari Monika; Christoph Weiste, Wolfgang Dröge-Laser

The onset of plant life is characterised by a major phase transition. During early heterotrophic seedling establishment, seed storage reserves fuel metabolic demands, enabling the subsequent transition to an autotrophic lifestyle. Whereas metabolic pathways leading to storage compound mobilization are well-described, the regulatory circuits remain largely unresolved. Using an inducible knockdown approach of the evolutionarily conserved energy master regulator SnRK1 (Snf1-RELATED-PROTEIN-KINASE1), transcriptomic studies reveal its crucial function in Arabidopsis seedling establishment (1). The downstream C/S1 bZIP (BASIC LEUCINE ZIPPER) transcription factor network (2) controls gene expression leading to amino acid catabolism to support energy-demanding processes. Moreover, they directly regulate cyPPDK (cytosolic PYRUVATE ORTHOPHOSPHATE DIKINASE encoding an important bottleneck enzyme to fuel gluconeogenesis (1). Based on transcriptome studies using SnRK1 knockdown approaches, we identified the SnRK1-dependent bZIP transcription factors DHP1/2 (DARK HYPOCOTYL PHENOTYPE1/2). Using knockout approaches, we confirmed their metabolic function in lipid catabolism, e.g., regulating the ACX4 (ACYL-COENZYME A OXIDASE4 (ACX4) gene. Whereas higher-order C/S1 bZIP factor mutants did not show phenotypical effects on germination and seedling establishment, we observed retarded germination and partially impaired hypocotyl elongation during etiolated growth of *dhp1/2*. Phenotypical and functional analyses are presented.

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- (1) Henninger M et al. 2021. Plant Cell 34:616-532.
- (2) Dröge-Laser W and Weiste C 2018. TIPS 23:422-433

8.Oct, 10:00-10:15



Aybaran Olca Kebabci

Actin conformation dynamics precede force generation in cytotoxic T lymphocytes

Adam M Rochussen, Aybaran O Kebabci, Alex K Winkel, Kristian Franze, Daniel A Fletcher, Gillian M Griffiths, Anna H Lippert

Cytotoxic T lymphocytes (CTLs) are cells of the adaptive immune system that are able to recognise and kill cancerous or virally infected target cells. The biochemical basis of CTL-mediated killing has been examined in great detail. Recent work has underscored the importance of physical forces in T cell function too, but how CTLs sense and exert force during migration and killing is not fully understood. Here, by expressing actin conformation probes based on the CH domain of utrophin, we directly visualize regions of altered F-actin conformation in primary CTLs during migration and killing. By combining these probes with traction force microscopy, we correlate external force with the internal cytoskeleton. We find strong, forward-directed traction forces at the leading edge of migrating CTLs that correlate with regions dominated by a lamellipodium-enriched actin conformation, and show that inhibition of non-muscle myosin IIa, Arp2/3, or formins disrupts force production without disrupting this biased actin conformation, indicating that conformation is regulated upstream of force. At the cytotoxic immune synapse, force exertion peaks 50-100 seconds after an early, force-associated actin conformation dominates the synapse, with this pattern conserved in physiological CTL-target cell conjugates. We show that actin conformation is regulated upstream of force production, and that force exertion at the cytotoxic immune synapse temporally follows actin conformation dynamics. Our work offers insight into the relationship between cellular force production and F-actin conformation in important primary immune cells.

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Judith Gabel

Science Communication Lab
Deutsches Museum, München

Dr. Judith Gabel studied physics in Würzburg and Stony Brook (U.S.) and subsequently earned her Ph.D. in experimental physics in Würzburg. She then spent three years conducting research as a postdoc in England before moving to Munich, where she shifted her focus more toward science communication: In a joint project between LMU and TU Munich, she developed courses on quantum technologies for industry and sought ways to convey this complex physics in an accessible manner. Her move to the Deutsches Museum marked her full transition into science communication: There, she heads the Science Communication Lab, which serves as a forum for discussing current research as well as a testing ground for new science communication formats.



Dennis Eckmeier

Science Journalist
Doktor Watson

Dennis works as a science journalist for one of the largest German speaking science channels on YouTube, 'Doktor Watson'. The channel received several awards for journalism and science communication, including the Grimme online Audience Award and Sartorius LifeScienceXplained. Dennis writes about a variety of topics in science and technology.

He received his biology degree at the University of Cologne in 2006 and a PhD in neuroscience at Bielefeld University in 2010. He worked as postdoc at Cold Spring Harbor Laboratory, USA, and at Champalimaud Research, Portugal until 2018



Barbara Hissa

Managing Editor
Beilstein Journal of Nanotechnology

Barbara Hissa graduated in Physics and obtained her Master's and PhD degrees in Cell Biology, from the Universidade Federal de Minas Gerais, Brazil. Barbara also did a postdoc in biophysics at the University of Chicago. After that, she moved to Germany to work as Deputy Lab Head in the Surgical Department of the University Hospital Mannheim investigating metastasis mechanisms. She then transitioned to the Beilstein-Institut as Managing Editor for the *Beilstein Journal of Nanotechnology*. Her current interests include: diamond open access model, research integrity, research inclusion and diversity, fair research assessment, science policy, nanotechnology, nanoscience, science communication.

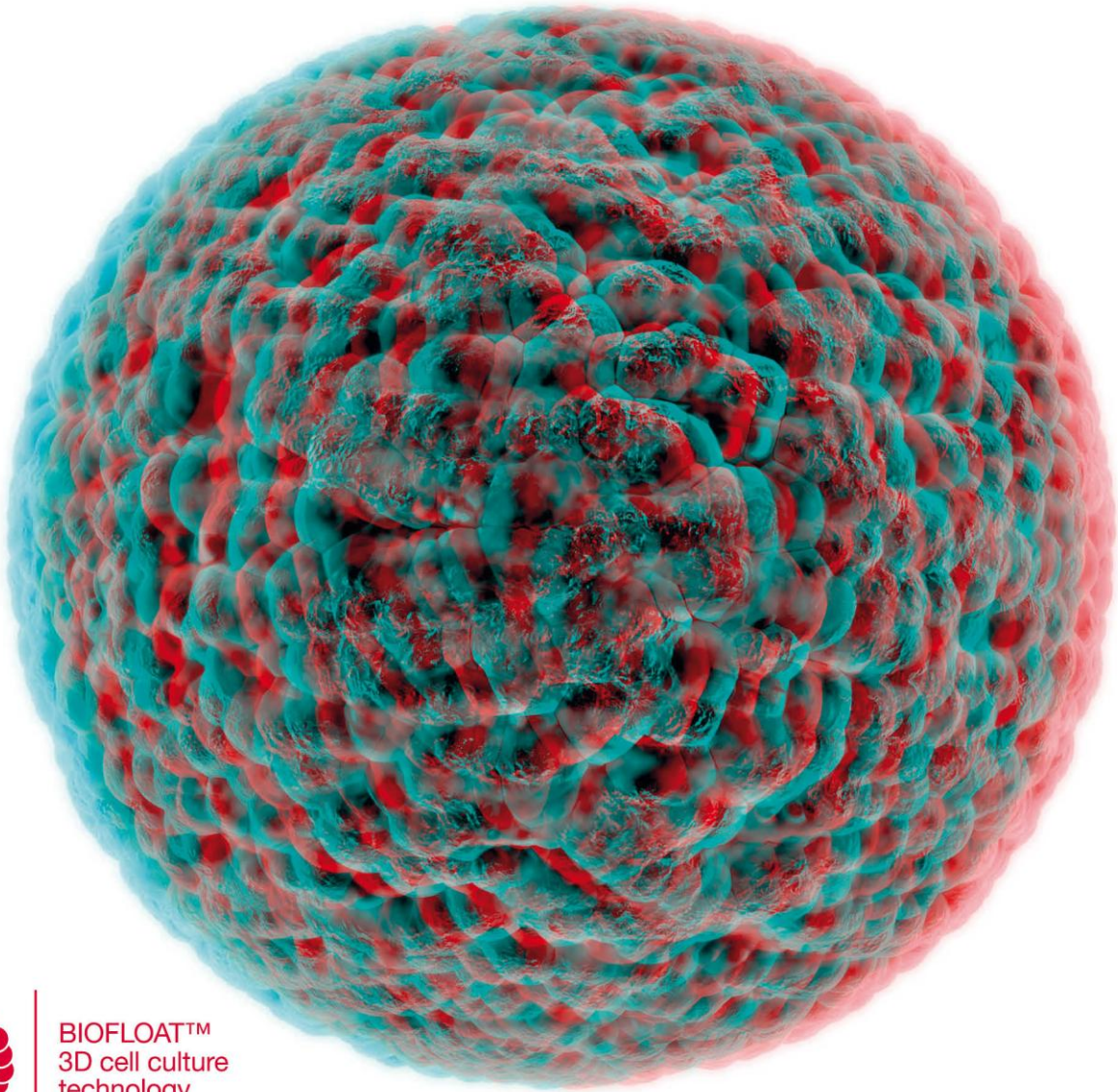


Tobias Kerrinnes

Scientific Infrastructure Unit
HIRI

Tobias Kerrinnes is a trained microbiologist with affiliations at the Robert Koch Institute, the University of California, Davis, and the Helmholtz-Center for Infection Research. After joining the Helmholtz Institute in Würzburg (HIRI), he served as lab coordinator, interim core facility head, and interim administrative head of the institute. Since May 2024, he has led the scientific infrastructure unit, working to create an excellent environment for scientists at the Helmholtz Institute.

His career reflects a journey from web lab scientist to a vital liaison connecting science and administration. This role involves engaging deeply with science, understanding the everyday needs of scientists, technologies, facility management, and administrative processes. The goal is to anticipate future developments and create the most supportive environment for every scientist to thrive in their research.



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POSTER SESSION



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Poster 1

Spatial Transcriptomics Identifying Restrictive and Permissive Macrophage Niches in Native Tissue during Intracellular Bacterial Infection

Parham Abedini, Camilla Ciolli Mattioli

Interactions between pathogenic bacteria and mononuclear phagocytes during early systemic infection determine whether the host clears the invading pathogen or infection becomes established. This outcome does not arise from a uniform host-pathogen encounter, but from the dynamic interplay between heterogeneous host niches and a phenotypically diverse bacterial population. Resolving this bidirectional heterogeneity requires methods capable of preserving both molecular identity and spatial context. A requirement not met by conventional tissue-dissociation-based single-cell approaches, which discard positional information.

This work applies spatial transcriptomics to dissect the transcriptional states of host immune cells and intracellular bacteria directly within infected tissue, in order to identify the cellular phenotypes that distinguish restrictive from permissive infection niches. *Salmonella enterica* serovar Typhimurium, a Gram-negative intracellular pathogen capable of invading and replicating within host macrophages, was selected as the bacterial model. Single-molecule and multiplexed FISH-based methods (smFISH and seqFISH) will be deployed on tissue sections from mice with systemic infection to map host and bacterial transcriptional states. Targeted seqFISH panels will subsequently be complemented with an unbiased, capture-based whole-transcriptome readout of host cells, enabling detection of infection-associated cell states that a predefined probe panel might otherwise miss.

By resolving where distinct bacterial phenotypic states localize relative to restrictive and permissive macrophage niches, this project aims to provide a spatially resolved framework for understanding how host-pathogen interactions and heterogeneity shapes the trajectory of infection.

Poster 2

Unbiased immune-neuro-vascular cellular profile in the skin of patients with complex regional pain syndrome

Eva Achhammer, Beate Hartmannsberger, Marc Schmidt, Julia Metz

Complex regional pain syndrome (CRPS) is a rare chronic pain disorder which typically affects one limb and is initialized by trauma, such as bone fracture or surgery. Patients experience severe, ongoing pain in the form of allodynia and/or hyperalgesia, as well as impaired function of the affected limb. Additional symptoms include vascular disturbances, that often appear as temperature asymmetry accompanied by edema and changes in skin color. Currently, only symptomatic treatments like pain medication, steroids, and physical therapy, are available. CRPS is described as an interaction between the immune, vascular and nervous system, which can best be analyzed in the skin. Previous studies have shown elevated levels of proinflammatory cytokines and neuropeptides (1). The number of Langerhans and mast cells is also increased (2), while the number of papillary microvessels is reduced (3). The underlying pathomechanism remains unclear.

Therefore, our goal is to study skin samples from the ipsilateral (affected) and contralateral (not affected) limbs of CRPS patients using single nuclei RNA sequencing (snRNAseq) to obtain an unbiased overview of all cell types involved. Thus far, samples from two patients have been analyzed, showing mainly changes within fibroblasts and keratinocytes. The data indicate an upregulation of cellular stress, extracellular matrix (ECM) remodeling, and inflammatory pathways on the ipsilateral side. To confirm these findings and also reliably identify changes within the smaller immune and endothelial cell populations, more patient samples have to be sequenced. and additional healthy control samples will be included. For further validation of the sequencing results, immune fluorescence staining and other in vitro experiments are planned. This project will thus contribute to our understanding of CRPS pathogenesis.

(1) Andronic et al., Rheumatology International ,2022; (2) Hartmannsberger et al., Journal of Neuroinflammation, 2024; (3) Mehling et al., Pain, 2024.

Poster 3

VEGFR1 controls intestinal pathology in allogeneic stem cell transplantation

Hla Ali¹, Hendrik Bartolomaeus¹, Sarah Schäfer¹, Maja Bundalo¹, Haroon Shaikh², Michael Kern², Emely-Sophie Diem², Andreas Beilhack², Alma Zerneck-Madsen¹ Institution (s): ¹Institute for Experimental Biomedicine, University Hospital Würzburg, Germany; ²Department of Internal Medicine II, University Hospital Würzburg, Germany

Endothelial dysfunction contributes to intestinal graft-versus-host disease (GvHD) following allogeneic stem cell transplantation (alloSCT). The exact mechanisms underlying these observations remain largely unclear. We used a novel mouse model (Flt1In13pA) in which a targeted mutation of an alternative polyadenylation site drives expression of soluble VEGFR1, sequestering VEGF-A, in the absence of membrane VEGFR1. Flt1In13pAmice showed improved vascular and epithelial integrity, reduced recruitment of activated allogeneic T cells to the colon and decreased inflammatory cytokine production. 16S rDNA sequencing revealed distinct gut microbial communities, with an increase in *Lactobacillus* spp. in Flt1In13 mice; the functional relevance of this is under investigation. Together, these data identify VEGFR1-dependent endothelial signaling as a key driver of intestinal pathology in alloSCT, affecting intestinal integrity and microbiome composition.

Poster 4

Optimized DNA-PAINT imaging for multiplexed cell biology in Gram-negative bacteria

Kilian Andress, Andreas Diepold, Christoph Spahn

Bacteria are highly organized organisms that orchestrate cellular processes in a spatiotemporal manner [1]. These processes, e.g DNA-replication and -segregation, cell wall synthesis or protein expression, are often physically coupled, yet this coupling remains understudied [2]. Reasons might be the transient nature of the underlying interactions, the small size of bacterial cells, as well as the increased molecular crowding compared to eukaryotic cells. DNA-PAINT is the ideal choice for nanoscale imaging of multiple cellular targets due to its superior resolution and multiplexing capability [3]. However, strand-selection and imaging conditions deviate from eukaryotic cells and need to be optimized accordingly. In this work, we present an RNA-FISH-based screening approach that enables cheap and straight-forward testing of different docking-imager-strand pairs in the Gram-negative bacterium *Escherichia coli*. We use the 16S rRNA-directed FISH probe EUB338 [4] carrying docking sequence overhangs as well as a scrambled version as control for signal specificity. Using this approach, we identified DNA-PAINT sequences suitable for 3D SMLM and quantified parameters such as relative on- and off-rates, localization precision and specificity. This information allows us to provide guidelines for high-quality DNA-PAINT in *E. coli*. Finally, we showcase the application of multicolor DNA-PAINT in super-resolution both on protein targets in *E. coli* and multicolor mRNA-FISH in the Gram-negative pathogen *Yersinia enterocolitica*. We hope that our work promotes the use of DNA-PAINT in bacterial cell biology.

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Poster 5

Effects of photoperiod and clock mutation on the development of *Drosophila*

Chaithra Anoop, Julian Bigge, Benjamin Risse, Christian Wegener

Previous studies in *Drosophila melanogaster* using the alleles of period genes had dissected the clock-dependent contributions to development. We are currently investigating the neuroendocrine mechanisms linking the circadian clock, photoperiod, and hormonal signalling pathways that together shape larval developmental progression, metamorphosis timing, and adult body size. Using a custom automated hatching-box system that enables continuous, non-invasive monitoring of key developmental transitions of pupariation and adult eclosion, we are quantifying developmental timing in clock mutants and wild-type flies under three photoperiodic conditions: LD 12:12, constant light (LL), and constant darkness (DD). Preliminary observations suggest that photoperiod influences developmental rate, with differences emerging across lighting conditions and clock genotypes. Importantly, body weight and wing size appear largely unaffected across conditions, hinting that any photoperiodic effect on timing may be independent of general metabolism.

To identify the potential neuromessengers relaying circadian information to endocrine centres, we performed immunostaining for candidate neuropeptides in larval clock neurons. Of the peptides screened- AstA, AstC, DH44, DH31, ITP, NPF, and CCHa1- only AstC was found to localise in DN1 clock neurons. To further map receptor expression in clock neurons, PTTH neurons, and insulin-producing cells (IPCs), we are establishing a dual-reporter system using Clk856-LexA combined with receptor-T2A-LexA lines. The overall aim is to determine the relative contributions of direct light input and clock-dependent signalling to developmental timing, and to define the neuroendocrine circuit through which the circadian clock exerts its effects.

This project has received funding from the European Union's HORIZON 2023 research and innovation programme under the Marie Skłodowska-Curie grant nr. 101169474.

Poster 6

Effects of recombinant IL-22 and IL-17 on the respiratory epithelial barrier during infections with *Candida albicans* and *Staphylococcus aureus*

L. Arbinger, T. Köhler, A. Schöninger, G. Braune, O. Kurzai, N. E. Nieuwenhuizen

Introduction

Pneumonia is a severe and potentially life-threatening disease affecting people of all ages. Hospital-acquired-pneumonia is defined by its occurrence at least 48 h post hospitalization or within 3 months of hospital treatment, while community-acquired-pneumonia has its onset in the private setting. *S. aureus*-pneumonia is often found in hospital settings and can result in severe illness with extensive need of antimicrobial therapy. Although *Candida albicans* is often isolated from the respiratory tract, particularly in mechanically ventilated patients, it almost never causes pneumonia. Experiments in a mouse model from our laboratory have demonstrated that pulmonary pre-colonization with *C. albicans* reduces the severity and invasiveness of subsequent *S. aureus* infection, associated with increased production of IL-22 and IL-17.

Aim

To investigate the influence of rIL-22 and rIL-17 on human lung epithelial cells as the first line of defense against respiratory infections with *S. aureus* and *C. albicans*.

Methods

We cultured the Calu-3 cell line as a model of the proximal respiratory tract as well as the A549 cell line as a model of the distal respiratory tract and assessed adhesion and invasion by *S. aureus* and *C. albicans* after pretreatment of the cells with rIL-22 and rIL-17. We implemented liquid-liquid interface (LLI) models for both A549 cells and Calu-3 cells. In addition, an air-liquid interface model (ALI) was established using Calu-3 cells. Epithelial cell damage was quantified using LDH cytotoxicity assays and epithelial barrier integrity was assessed via TEER measurements. The epithelium was visualized using fluorescence microscopy and histological staining. IL-8 was measured by ELISA.

Results

We successfully established an ALI model using Calu-3 cells, with features such as pseudostratification and mucus production, increasing the physiological relevance of the infection model. TEER-measurements revealed a decrease in the epithelial tight-junction integrity after infection. No significant differences in TEER values or in *S. aureus* or *C. albicans* burdens were observed after pre-treatment with rIL-17 or rIL-22. Calu-3 cells had higher baseline secretion of IL-8 than A549 cells, and both rIL-17 and *C. albicans* infection increased secretion in Calu-3 cells. To further investigate how rIL-22 and rIL-17 affect lung epithelial cells, we are evaluating gene expression of antimicrobial peptides by qRT-PCR.

Poster 7

Selective TNFR2 agonists expand functional human regulatory T cells without activating conventional T cells

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TNFR2, a member of the TNF receptor superfamily (TNFRSF), plays a major role in modulating the immune response. In contrast to the TNF- α blockade, proved to be effective as cancer therapy, targeted stimulation of TNFR2 holds strong therapeutic potential for autoimmune inflammatory diseases. Hexameric TNFR2-selective TNF mutants protect mice against GvHD. Dodecameric agonists promise superior TNFR2 activation. Thus, we systematically tested novel TNF-based TNFR2 agonists with varying domain architectures and 6 to 12 binding sites for TNFR2 to determine their effects on human regulatory T cells (Tregs) and conventional T cells (Tcons).

To generate hexa-(LC, HC/NewSTAR2 Hel-based), nona-(hSTAR2), and dodecameric (GCN4, HCLC/NewerSTAR2) TNFR2 agonists, single-chain domains containing three TNFR2-specific TNF protomers were fused with oligomerization domains or an IgG1 antibody. The resulting constructs were tested in vitro by stimulating human PBMCs from healthy individuals (n=7) for 4 days. All tested constructs significantly increased FoxP3^{hi}CD25^{hi} Treg frequencies (Fold changes: LC: 1.84 P<0.05; HC/NewSTAR2 Hel-based: 2.20, hSTAR2: 2.23, GCN4:2.24, P<0.01; HCLC/NewerSTAR2: 2.48 P<0.001), while frequencies of CD4 Tcons and CD8 T cells remained unaffected. However, the differences between constructs were not significant. Stimulating human PBMCs for 4 days with agonist concentrations of 1 – 1000 ng/mL yielded dose-response curves. The data revealed high inter-donor variability, but Treg expansion generally followed a linear dose relationship for most agonists. Next, we performed allogeneic mixed lymphocyte reactions with enriched T cells and T cell-depleted fractions of PBMCs to investigate if the highest tested dose of TNFR2 agonists could act as costimulatory signals during TCR stimulation. Remarkably, adding the TNFR2 agonists did not significantly increase the proliferation of CD4 or CD8 Tcons, indicating little to no costimulatory effect. Even though FoxP3⁺ T cells showed higher proliferation and division indices than FoxP3⁻ T cells and CD8 T cells in some samples, we did not observe a clear construct-driven improvement in Treg expansion under these conditions. In a suppression assay, we added TNFR2-agonists (1000 ng/mL) after one day of coculturing Treg-depleted CD4 T cells and enriched Tregs with T cell-depleted PBMCs from an allogeneic donor. After 4 days, we consistently observed mildly reduced proliferation of CD4 Tcons in combination with enhanced proliferation of Tregs when the different TNFR2 variants were added to the coculture, with no significant differences between the treated groups. In summary, the TNFR2 agonists expand Tregs regardless of valency. In alloreactive settings, the agonists did not act as costimulatory molecules for alloreactive T cells. Since the positive immunomodulatory effects persisted at physiological Treg frequencies and after TCR stimulation, these novel TNFR2 agonists could potentially prevent and suppress pathogenic T cell responses.

Poster 8

Analyzing and Targeting Non-Catalytic Functions of Aurora-A in Acute Myeloid Leukemia.

Rishika Basak, Mareike Solvie, Prof.Dr. Elmar Wolf, Prof.Dr. Gabriele Büchel

Aurora-A is a serine/threonine kinase best known for its mitotic role in centrosome maturation and spindle assembly, and it is frequently overexpressed or amplified in cancer. Clinical development of catalytic inhibitors has been limited by toxicity, particularly off-target inhibition of Aurora-B, while accumulating evidence points to kinase-independent scaffolding functions that drive oncogenic signaling but are not addressed by conventional inhibitors. Aurora-A stabilizes MYC-family oncoproteins through such non-catalytic, protein-complex-based mechanisms across multiple cancer types. PROTAC-mediated degradation removes both catalytic and non-catalytic functions of Aurora-A and has shown potent anti-tumor activity, including S-phase arrest and apoptosis in AML models. This project aims to define the non-catalytic functions of Aurora-A in MYC-driven tumors, using AML as the primary model system, and to assess the therapeutic potential of Aurora-A degradation.

Neuroblastoma will serve as a supporting comparative model, since Aurora-A's RNA-binding and its S-phase phosphorylation of CDK12 have so far only been established there. A key aim is to determine whether this same axis operates in AML, using neuroblastoma findings as a benchmark for mechanistic comparison. We will characterize Aurora-A-MYC complex formation and its dependence on cell-cycle phase and cellular context and compare conventional kinase inhibition with PROTAC-mediated degradation to isolate scaffolding-specific effects. In vivo efficacy of Aurora-A PROTACs will be tested in AML models, alone and combined with ATR inhibition to exploit replicative-stress vulnerability. Mechanistically, we will assess whether degradation promotes transcription-replication conflicts, R-loop accumulation, and DNA damage in AML, testing whether the CDK12/RNA-binding-linked mechanism identified in neuroblastoma is conserved. RNA sequencing will distinguish degradation from inhibition-specific transcriptional effects in AML and nominate biomarkers, which will be validated by qPCR, histology, and proximity ligation assays.

Promising biomarkers will be evaluated in AML patient samples for stratification and therapeutic monitoring. By using neuroblastoma as a comparative model, this study will determine whether the CDK12/RNA-binding mechanism first described there is a conserved feature of Aurora-A biology or AML-specific, defining kinase-independent Aurora-A functions in MYC-driven cancers and establishing whether targeted degradation offers a therapeutic strategy beyond conventional kinase inhibition.

Poster 9

Evaluating ASOs as antibacterial agents for treatment of *C. difficile* infection

Christopher Birk, Franziska Faber

Although antibiotic treatment is still the main therapeutic option for *C. difficile* infections (CDI), it frequently results in disease recurrence and further exacerbates dysbiosis of the gut microbiome. Therefore, strategies aimed at interfering specifically with *C. difficile* survival and pathogenicity are earnestly needed. Synthetic nucleic acid analogues have recently emerged as a promising new strategy to target bacterial pathogens in a sequence-specific manner. These antisense oligonucleotides (ASOs) enable selective targeting of a bacterial species of interest within a complex community through ASO-mediated mRNA repression. Our study aimed to systematically evaluate peptide nucleic acid (PNA) based ASOs targeted against essential and virulence-associated genes in *C. difficile*.

We screened a library of more than 100 peptides for their capacity to deliver cargo across the *C. difficile* cell envelope using flow cytometry and super-resolution microscopy. The three lead peptides were combined with PNA ASOs targeting the translation initiation region (TIR) of essential and virulence-related genes. Using growth curves and CFU counting, one promising peptide-PNA was selected for optimization and RNA-seq.

A 10-mer PNA targeting the start codon region of RNA polymerase subunit α (*rpoA*) coupled to the TAT peptide shows bactericidal activity against a variety of *C. difficile* isolates from all 5 genomic clades. Transcriptomic response of the model strain 630 exposed to peptide-PNA suggests a mechanism of action at the posttranscriptional level, consistent with current understanding of ASO based gene silencing in other bacteria.

In this study, we designed and characterized a PNA-based antisense oligonucleotide capable of effectively killing *C. difficile*. Considering the urgent need for alternatives to classic broad-spectrum antibiotics, novel ideas and approaches to antimicrobials are highly relevant. The ASO presented here represents a novel class of targeted antimicrobial therapeutics that could fill this need. This study marks a first step in systematically evaluating the future use of asobiotics in CDI treatment.

Poster 10

Differential targeting of human GPVI provides mechanistic insight into inflammatory bleeding in the lung

S.I. Bonfiglio, S. Navarro, V. Orth, and B. Nieswandt

Glycoprotein (GP)VI is a platelet-specific collagen/fibrin(ogen) receptor and a promising target to treat thrombotic and thrombo-inflammatory disease states. Notably, previous studies proposed a key role of GPVI in maintaining vascular integrity under inflammatory conditions, although the underlying mechanisms are not fully understood. Different approaches have been developed to inhibit GPVI function *in vivo*, most notably receptor blockade by antibody Fab fragments and GPVI immunodepletion by intact IgGs.

We aim to mechanistically dissect the role of human (h)GPVI in vascular integrity under inflammatory conditions in a model of lipopolysaccharide (LPS)-induced acute lung injury (ALI) in mice.

hGPVI function and surface abundance was modulated *in vivo* in humanized GPVI (hGP6tg/tg) mice using three recently developed inhibitory strategies: complete or partial receptor depletion by high (Emf1) or low (JAQ1) affinity antibodies, respectively, as well as functional inhibition by Emf1Fab. ALI was induced by intranasal instillation of LPS in hGP6tg/tg mice. Broncho-alveolar lavage fluid (BALF) and lung cryosections were analyzed to assess LPS-induced inflammatory parameters and bleeding. Platelet function was assessed *ex vivo* in flow adhesion assays.

Following intranasal LPS challenge, mice treated with Emf1, JAQ1, or Emf1Fab displayed attenuated hypothermia. Immunofluorescent analysis of lung cryosections revealed decreased neutrophil infiltration and platelet-neutrophil complexes formation compared to LPS-treated controls. Diminished neutrophil content in the BALF indicated reduced extravasation in all treatment groups. Remarkably, JAQ1 and Emf1Fab, but not Emf1 treatment also significantly reduced BALF hemoglobin content. Finally, *ex vivo* flow adhesion assays revealed that while all treatment groups led to abolished collagen-dependent platelet activation in terms of phosphatidylserine-exposure, JAQ1 and Emf1Fab treatment preserved hGPVI adhesive function.

While hGPVI inhibition consistently attenuated pulmonary inflammation, targeting strategies that preserve the receptor's adhesive function also preserved vascular integrity during ALI, thereby minimizing bleeding complications.

Poster 11

Development of Covalent Sigma-1 Receptor Ligands for Application in (Photo)Pharmacology and Bioimaging

Luise Bühner

Alzheimer's disease (AD) represents a devastating neurodegenerative disease, leading to high costs for the society as well as very high burdens for both patients and their families. Treatments developed to date did not prove to be successful, but sigma-1 receptor (S1R) agonists were shown to lead to pronounced neuroprotective effects in mouse models and cognitive preservation in AD patients, making the S1R an interesting target for drugs against AD.

Building on these observations, the present project aims to develop covalent ligands for the S1R, which should introduce a stable chemical bond to the target protein and therefore facilitate the use of advanced assays and experimental methods, while simultaneously avoiding competition with endogenous ligands and increasing selectivity. Different warheads targeting tyrosine residues will be tested, including fluorosulfonates and sulfonyl triazoles, which work via sulfur-fluoride/triazole-exchange reactions, and cyclic imines, which react in a Mannich type reaction. To increase the chances for stable, selective, and active ligands, different positions on the considered parent ligands and different core lengths will be synthesised and evaluated.

Furthermore, introduction of a suitable fluorophore into the covalent ligand is envisaged to enable the development of a diagnostic tool for diseases like Parkinson or amyotrophic lateral sclerosis, which include a disintegrity of the mitochondria associated membrane (MAM). This can be done by correlative light and electron microscopy (CLEM), which has never been applied for investigating the subcellular location of the S1R so far, but with which the Decker group already has prior experience. To introduce fluorescence, either the ligand itself can be modified with a fluorophore in its core, or an alkyne handle will be added that allows the introduction of a fluorophore after binding to the cells via click chemistry.

If time allows it, the expertise of the Decker group regarding photoswitchable compounds will also be used to modify the covalent ligands further. Therefore, azobenzene units would be introduced to allow changes in the affinity with very high spatial but additionally also temporal control and thereby turn the native S1R into an artificial photoreceptor.

Poster 12

Shining Light on Hemostasis and Thrombosis - Photopharmacology on FXa

Ariel Cedillo

Thrombosis is a common pathology that triggers myocardial infarctions, strokes and venous thromboembolism. It is heavily related to other pathologies as atrial fibrillation, coronary artery disease and atherosclerosis, heart failure, and certain types of cancer. The current treatment for thrombosis involves the inhibition of platelet activity, fibrinolysis activation, and inhibition of coagulant factors. The latter inhibition is achieved through parenteral administration of low molecular weight heparin, oral vitamin K antagonists such as warfarin, and direct oral anticoagulants (DOACs). The main mode of action of DOACs is the inhibition of thrombin or factor Xa (FXa). Despite the safety and effectiveness of the clinically approved DOACs, they still can trigger uncontrolled bleeding upon injuries. Therefore, the activity modulation with high spatial and temporal specificity given by photopharmacological approaches becomes a promising improvement for the current thrombosis treatment.

Over the years, photopharmacology has provided different molecular tools for the modulation of GPCR activity. However, the enzymatic activity involved in hemostasis modulated via small molecule photoswitchable ligands has not been reported. On this context, the current project aims the design, synthesis and evaluation of photoswitchable FXa inhibitors. Having as main goal, photoswitches with short thermal half-lives that can be isomerized in the visible wavelength range 380 nm (violet) – 750 nm (red).

Poster 13

Deep Brain Stimulation decreases alpha- synuclein pathology and immune cell reactivity in a mouse model of Parkinson's disease

Wei Chen, Rhonda McFleder, Jens Volkmann, Chi Wang Ip

Aims:

To investigate whether chronic subthalamic nucleus deep brain stimulation (STN-DBS) attenuates pathological α -synuclein aggregation in a mouse model of α -synucleinopathy.

Methods:

Wild-type mice received stereotactic injections of an adeno-associated virus serotype 1/2 (AAV1/2) expressing human mutant α -synuclein (A53T) into the substantia nigra (SN). Two weeks after injection, electrodes were implanted into the subthalamic nucleus (STN), followed by continuous DBS for 12–14 days. At six weeks post-injection, brains and spleens were collected. Tyrosine hydroxylase (TH)-positive dopaminergic neurons, T-cell infiltration, α -synuclein phosphorylation (pS129 α -synuclein) and α -synuclein aggregation were assessed by immunohistochemistry and immunofluorescence. Splenocytes were isolated and stimulated with α -synuclein peptides for two weeks, and α -synuclein-specific immune reactivity was assessed by IFN- γ ELISpot. Motor function was evaluated using the open field and cylinder tests.

Results:

In the substantia nigra pars compacta (SNpc), STN-DBS reduced A53T-induced loss of TH-positive dopaminergic neurons. Importantly, pathological α -synuclein aggregation, pS129 α -synuclein, and T-cell infiltration in the SNpc were significantly reduced following DBS. Furthermore, the increased reactivity of splenocytes to α -synuclein peptides observed in A53T mice was attenuated following DBS, as indicated by reduced IFN- γ responses

Conclusions:

STN-DBS attenuates pathological α -synuclein aggregation and phosphorylation and reduces α -synuclein-associated immune responses in a mouse model of α -synucleinopathy. These findings suggest that modulation of α -synuclein pathology and neuroimmune responses may contribute to the neuroprotective effects of STN-DBS and support further investigation of DBS as a potential disease-modifying intervention for Parkinson's disease.

Poster 14

Targeting neuroblastoma using Aurora-A inhibition in combination with CAR T-cells.

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Amplification of the growth-promoting oncogene MYCN is a driving mutation in a subpopulation of neuroblastoma patients and is associated with poor clinical outcome. During S phase, MYCN is stabilized through complex formation with the Aurora-A. Pharmacological inhibition of Aurora-A with MLN8237 (Alisertib) can disrupt this complex; however robust antitumor activity requires relatively high drug concentrations (Brockman et al., 2013). Combined Aurora-A and ATR inhibition significantly prolonged the survival of mice harboring a neuroblastic tumor. In a subset of animals, this regimen induced durable tumor regression and eradication through both tumor cell-intrinsic and immune cell-mediated mechanisms (Roeschert et al., 2021). These findings suggest that the immune response elicited by the combination could be leveraged to develop rational combination strategies with immunotherapies for neuroblastoma.

We characterized LY3295668 (LY668), a novel, on-target Aurora-A inhibitor. In neuroblastoma cells, LY668 treatment induced cell cycle arrest and apoptosis. Although our previous work showed that Aurora-A inhibition enhance immune cell infiltration into the solid tumor, it also reduced T-cell viability. These opposing effects motivated the development of a strategy that preserves the tumor-sensitizing effects of Aurora-A inhibition while protecting CAR T-cell function. To evaluate the therapeutic potential of CAR T-cell therapy in neuroblastoma, we pursue two complementary strategies: (I) combining Aurora-A inhibition with CAR T-cells and (II) engineering CAR T-cells that are resistant to Aurora-A inhibitors. Aurora-A inhibitor-resistant CAR T-cells were generated by introducing an inhibitor-resistant Aurora-A variant.

In co-culture assays, CAR T-cells completely eradicated tumor cells. However, co-treatment with LY668 impaired CAR T-cell function, resulting in the persistence of residual tumor cells. In contrast, Aurora-A inhibitor-resistant CAR T-cells retained their antitumor activity under Aurora-A inhibitor treatment. Similar results were obtained in a rechallenge co-culture assay, in which both CAR T-cell populations were repeatedly exposed to tumor cells in the presence of an Aurora-A inhibitor.

These findings provide a rationale for combining Aurora-A inhibition with Aurora-A inhibitor-resistant CAR T-cells to achieve complete tumor eradication and to prolong event-free survival in treated mice.

Poster 15

Splicing-Derived Tumor Antigens as Targets for TCR-based Immunotherapy in Medulloblastoma

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Medulloblastoma (MB) is the most common malignant brain tumor in children and comprises four molecular subgroups: WNT, SHH, Group 3 (G3) and Group 4. MYC-amplified G3 MB is characterized by an aggressive clinical course and poor prognosis, while current treatments remain largely non-subgroup-specific. Moreover, MB displays a low mutational burden, limiting the availability of mutation-derived tumor-specific antigens (TSAs). Aberrant RNA splicing may therefore represent an alternative source of tumor-specific peptides and an exploitable target for immunotherapy. We aimed to identify splicing-derived TSAs in high-risk G3 MB and evaluate their potential for TCR-based immunotherapy. RNA-seq data from 16 G3 MBs were compared with fetal (n=5) and adult (n=4) healthy cerebellum samples. We identified thousands of differentially regulated alternative splicing events, approximately half of which were not annotated in FAST-DB and were predominantly intronic. Focusing on recurrent upregulated intron retention (IR) events, we predicted MHC class I-binding peptides and applied a multistep selection based on statistical significance, recurrence, tumor specificity, translational potential and predicted immunogenicity. This strategy identified 23 candidate antigens arising from recurrent IR events.

RT-PCR validation in G3 MB samples confirmed tumor-specific expression of 11 IR events. Subcellular fractionation and polysome profiling further identified six candidates (PSD, CLDN10, COX7A1, APC2, LCAT and ABTB2) with cytoplasmic localization and association with actively translating polysomes, supporting their potential to generate immunogenic peptides.

To assess their immunogenic potential, naïve CD8⁺ T cells were primed ex vivo with peptide-loaded mature dendritic cells and expanded with IL-7 and IL-15 to generate peptide-specific CTLs. Their responses were evaluated by IFN- γ ELISpot using peptide-pulsed HLA-A2⁺ K562 cells as antigen-presenting cells. CLDN10, COX7A1 and PSD elicited peptide-specific IFN- γ responses, with increased responses following repeated stimulation. Peptide-MHC dextramer staining is being used to enrich antigen-specific CD8⁺ T cells for single-cell sorting and TCR sequencing, enabling identification of dominant peptide-specific TCR clonotypes. Further work will focus on selecting specific TCRs, which will be cloned and functionally validated against identified MB-derived peptides in vitro and in vivo. Together, our findings identify aberrant IR as a potential source of tumor-specific antigens in G3 MB and provide a framework for the identification of antigen-specific TCRs, laying the groundwork for personalized TCR-based immunotherapy for high-risk MB

Poster 16

Role of TRIM28 in kidney development and the formation of pediatric kidney tumors

Greta Clarén, Matthias Becker

Wilms tumor represents one of the most common childhood malignancies and is also the most common pediatric renal tumor. Wilms tumor is thought to arise from multipotent embryonic kidney precursor cells due to mutations of genes involved in kidney differentiation. Among these genes are many epigenetic modifiers such as TRIM28.

TRIM28 acts as a transcriptional corepressor, e.g. by mediating chromatin condensation at transposable element (TE) loci, but the exact mode of action and its function in development is not yet fully understood. To gain a better understanding of the role of TRIM28 in kidney development we employ a mouse embryonic stem cell (ESC) differentiation paradigm which allows the differentiation towards metanephric mesenchyme and nephric epithelial cells. To shut down TRIM28 at different time points during in vitro renal differentiation we use ESCs that encode endogenously tagged degradation-sensitive TRIM28-FKBP alleles, where the TRIM28 protein can be depleted upon addition of the dTAG-13 ligand. Depleting TRIM28 in this in vitro system resulted in an increased expression of transposable elements and an increase in the frequency of E-Cadherine-positive epithelial cells. These results are similar to the results we obtained from a conditional TRIM28 knock-out mouse model system, which also shows increased expression of TEs and an increased frequency of epithelial cell types upon Trim28 deletion.

In order to gain a better overall understanding of the role of TRIM28 in human kidney organogenesis and tumorigenesis, a differentiation paradigm based on human induced pluripotent stem cells (hiPSC) was established. Our results show an effective differentiation of these cells towards mature kidney structures such as glomeruli, which are defined by double positive staining for the podocyte markers WT1 and podocalyxin. To investigate the effect of TRIM28 loss, hiPSCs were modified to express a degradable FKBP-tagged TRIM28. This system will be instrumental to study the role of TRIM28 during human in vitro kidney differentiation.

Poster 17

Jejunal IL-22–STAT3 activation mediates systemic anti-inflammatory remodeling after Roux-en-Y gastric bypass

Philip Claus, Maximilian Gruber, Rebecca Springer, Florian Seyfried, Christoph Otto, Nicolas Schlegel

Background

Roux-en-Y gastric bypass (RYGB) is the most effective therapy in morbid obesity, inducing sustained weight loss and metabolic improvements, but the underlying mechanisms remain unclear. Since obesity features chronic low-grade inflammation, we hypothesized that RYGB effects can partly be explained by modulation of intestinal cytokine signaling independent of caloric restriction effects.

Methods

Male Wistar rats with high-fat diet (HFD)-induced obesity were randomized to sham surgery (sham), sham surgery pair-fed to RYGB (PF), or RYGB (n = 28). Age-matched low-fat-diet (LFD)-fed animals served as lean controls (n = 6). Multi-modal phenotyping included bulk RNA-sequencing of jejunum and colon, 15-plex Luminex cytokine profiling of portal vein and systemic blood, 16S rRNA microbiome analysis, junctional protein Western blot, and histology.

Results

RYGB and PF rats showed weight loss and improved glucose tolerance compared to obese sham. Luminex profiling revealed strongly elevated IL-22 and IL-10 in both portal and systemic circulation of RYGB, while pro-inflammatory cytokines (IL-6, IL-1 β , TNF) were mildly elevated in portal blood, indicating an anti-inflammatory systemic signature. Histologically, RYGB induced jejunal and ileal crypt-villus hyperplasia and ileal macrophage accumulation. Accordingly, bulk mRNA-sequencing followed by gene set enrichment analysis (GSEA) revealed a strong response of IL-22/STAT3 target genes in RYGB, including antimicrobial peptides Reg3b (log₂FC = +4.7) and Reg3g (+5.0), with downregulation of the IL-22 decoy receptor Il22ra2. This was confined to the jejunum but not the colon, indicating region-specific effects. Functionally, intestinal epithelial barrier reorganization was evident by normalization of systemic serum LPS, with a RYGB-specific increase of portal LPS. As revealed by 16S sequencing, gut microbial changes were distinct from PF, with a strong increase of LPS-producing *Clostridium_P* and *Escherichia*.

Conclusions

RYGB triggers a tissue-restricted IL-22/STAT3 program in jejunum that translates into systemic anti-inflammatory cytokine signatures. These findings identify IL-22 as a potential effector of bariatric surgery-induced metabolic improvement and suggest IL-22 pathway modulation as a promising surgery-mimetic strategy.

Poster 18

Photoswitchable anticoagulants and antiplatelet agents for local modulation of hemostasis and thrombosis

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Background: Current antiplatelet and anticoagulant therapies are highly effective in preventing thrombotic events and have become indispensable in clinical therapy; however, their positive profile is limited by the inherently increased bleeding risk. This emphasizes the need for developing safer strategies that preserve the antithrombotic effect while mitigating hemorrhagic complications. The incorporation of a photosensitive moiety into already established medication can enable controlled inactivation of the pharmacological effect with high spatial and temporal precision, offering a promising solution to this limitation.

Aims: We aim to evaluate the antiplatelet and anticoagulant properties of novel photoswitchable compounds in murine models (in vitro/in vivo) and in human blood.

Methods: A photosensitive moiety was incorporated into already established antiplatelet/anticoagulation compounds, enabling their (in)activation by precise light exposure. Monochromatic light with a wavelength of 356nm induces a switch towards the cis form for both compound types, while 475nm or 530nm isomerizes them to their trans state. In vitro assays were performed using either human plasma or mouse blood to characterize the anticoagulant and antiplatelet compounds. These assays range from coagulation assays using plasma platelet and activation/aggregation assays using purified platelets, to assays utilizing whole blood in a rotational thromboelastometry setup, enabling measurements for clotting time.

Results: Photophysical and photochemical characterization confirmed stable and fast switching between both cis and trans photo-isomers. Anticoagulant compounds targeting coagulation factor X (FX) showed pronounced light dependent reduction of FX activity and fibrin time in human PPP, as well as inhibition of human blood clotting for the cis isomer. Antiplatelet agents targeting the major platelet integrin $\alpha IIb\beta 3$ proved light-dependent modulation of platelet aggregation in response to collagen using both human and mouse washed platelets in the trans configuration.

Outlook: Subsequent work will extend to in vitro models for thrombus and fibrin formation (whole blood in a coagulation flow chamber) as well as in vivo models of thrombus formation (FeCl₃-induced injury of mesenteric arterioles, mechanical injury of abdominal aorta) and hemostasis (tail bleeding assay) aiming to establish a proof of concept for spatiotemporally controlled photoswitchable antithrombotic therapy.

Poster 19

Large Extracellular Vesicles as mediators of blood-brain barrier dysfunction during *Neisseria meningitidis* Infection

Mathis Dreher, Alina Weinmann, Fabian Schumacher, Dominik Wigger, Burkhard Kleuser, Alexandra Schubert-Unkmeir

Large extracellular vesicles (IEVs) are plasma membrane-derived vesicles that mediate intercellular communication by transferring proteins, lipids, and nucleic acids, and are increasingly recognized as biomarkers and therapeutic targets in a variety of diseases. *Neisseria meningitidis* (Nm) is a human-specific, gram-negative diplococcus that asymptotically colonizes the human nasopharynx but can cause invasive diseases such as meningitis and septicemia.

We recently found that Nm infection of human brain endothelial cells (hCMEC/D3) leads to inhibition of dihydroceramide desaturase (DEGS) activity, causing intracellular accumulation of dihydroceramide (dhCer) and its redistribution to the Golgi apparatus and plasma membrane, compartments central to IEV biogenesis. We therefore hypothesized that dhCer is actively incorporated into IEVs and contributes to a biological activity during infection. IEVs were isolated from infected and uninfected hCMEC/D3 cells by ultracentrifugation and density gradient fractionation. To exclude contamination with bacterial outer membrane vesicles (OMVs), only fractions 1-5 were used, and IEV identity was confirmed by Integrin β 1 detection prior to pooling. Lipid composition, including dhCer species, was quantified by LC-MS/MS. hCMEC/D3 cell monolayers were treated with IEVs from infected and uninfected cells, and barrier function, tight junction proteins (TJP) localization, and transcriptional changes were assessed by ECIS, confocal microscopy, and qPCR. IEVs from fractions 1-5 impaired barrier functions and reduced electrical resistance in hCMEC/D3 monolayers, accompanied by apparent destabilization of the TJP ZO-1 on confocal microscopy. Lipidomic profiling identified only one dhCer species, 24:1 dhCer, as significantly enriched in IEVs from fractions 1–5, suggesting that dhCer alone is unlikely to account for the observed barrier-disruptive effects. To further evaluate and classify the biological role of IEVs released during infection with Nm, qPCR and ELISA measurements will be conducted.

In summary, IEVs released from brain endothelial cells during Nm infection disrupt blood-brain barrier integrity and represent a potentially important target for both therapeutic intervention and a deeper mechanistic understanding of meningococcal pathogenesis.

Poster 20

Benchmarking interpretable representation learning for phenotypic microscopy

Mingcheng Fan, Zongwei Wu, Christophe Zimmer

Deep learning has substantially advanced image-based phenotypic discovery by enabling the extraction of rich representations from microscopy images. However, it remains unclear whether these representations capture distinct phenotypic factors that are meaningful for biological interpretation. Existing explainability methods, such as gradient-based activation mapping, primarily identify relevant image regions rather than revealing how learned representations encode underlying phenotypic factors. Hence, in this work, we propose to establish a systematic benchmark for representation interpretability based on disentangled representation learning (DRL), which is designed to separate distinct factors of variation within learned representations and may therefore provide a more direct route to biologically meaningful interpretation.

Specifically, we develop 2 synthetic microscopy datasets in which individual phenotypic and imaging factors can be precisely controlled and independently varied, including cell morphology, and imaging conditions. The controlled settings provide explicit ground truth for assessing whether learned representations recover and separate these underlying factors. Based on these controlled datasets, we systematically compare multiple deep learning and representation-learning paradigms using an established disentanglement metric.

Our benchmark reveals substantial differences between predictive performance and representation interpretability. Across both datasets and across predictive and generative modelling paradigms, exemplified by variational autoencoders (VAEs) and conventional supervised convolutional neural networks, ICA shows the highest interpretability. These findings suggest that learning representations without optimizing for a specific predictive task may better preserve distinct phenotypic factors, providing a foundation for more interpretable image-based phenotypic discovery.

Poster 21

Mechanisms of Nuclear m6A RNA Deposition and Their Impact on Gene Regulation

Kiara Gäberlein, Julian König

N6-methyladenosine (m6A) is one of the key regulatory modifications of mRNA and influences almost every aspect of mRNA metabolism, from expression and pre-mRNA processing in the nucleus to translation and mRNA decay in the cytoplasm. m6A methylation is dynamic as well as reversible and often deposited co- transcriptionally. It is usually present in a sequence specific motif throughout transcripts and enriched around stop codons in long exons and after the last splice site. In eukaryotic cells, RNA m6A level is dynamically regulated by "writers" and "erasers", and recognized by "readers" in direct or indirect ways.

The planned work will investigate the molecular mechanisms of nuclear m6A deposition, including spatial organization, the protein complexes involved and regulatory signals. By combining biochemical, cell biological and genomic methods, the aim is to develop a comprehensive model that explains how m6A marks are controlled in the cell nucleus and what functional consequences this has for gene regulation.

Poster 22

Inhibition of GLUT1 sensitizes multiple myeloma cell lines to conventional PIs and melphalan

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Multiple myeloma (MM) is a clonal plasma-cell neoplasm characterized by the uncontrolled proliferation of malignant plasma cells (PCs) within the bone marrow, typically accompanied by M-protein secretion and end-organ damage. Among the metabolic features that distinguish malignant from normal cells is a constitutively enhanced glucose uptake. Glucose entry into mammalian cells is mediated by the facilitative glucose transporter (GLUT) family proteins, encoded by the SLC2A genes, which comprises 14 structurally related family members (GLUT1-14). Across several malignancies, GLUT1 overexpression has been associated with reduced overall and disease-free survival, supporting its potential value as both a diagnostic and prognostic biomarker. Despite recent therapeutic advances, resistance to proteasome inhibitors (PIs) and conventional chemotherapeutic agents continues to represent a clinical challenge. Given the reliance of tumor PCs on glycolytic metabolism to sustain proliferation and survival, targeting GLUT1 emerges as an attractive strategy to disrupt this metabolic dependency. In MM, further characterization of GLUT1 and its potential as a therapeutic target may uncover novel avenues to overcome drug resistance and improve patient outcomes. Using conventional 2D in vitro cultures, this study aimed to evaluate the effect of the GLUT1 inhibitors BAY-876 and STF-31 on cell viability across MM cell lines such as AMO-1, INA-6, JIN3, and RPMI 8226, as well as the proteasome inhibitor (PI)-resistant cell lines AMO-1R180-IXA and INA-6R17-CAR, both alone and in combination with bortezomib (BZM), carfilzomib (CFZ), ixazomib (IXZ), or melphalan (MEL).

Cell viability was determined by annexin V-FITC/PI staining using an Attune CytPix flow cytometer (Thermo Fisher Scientific), with data analysis performed in GraphPad Prism. Both BAY-876 and STF-31 significantly reduced the percentage of viable cells relative to the DMSO control at 24 and 48 hours across all conventional MM cell lines tested ($p < 0.0001$). STF-31 displayed a markedly stronger cytotoxic effect at high doses (30–100 μM) after 48 hours compared with BAY-876. Combining BAY-876 or STF-31 with bortezomib (BZM: 9.76–30.57 nM) or melphalan (MEL: 49.15 μM), resulted in a significant, dose-dependent increase in cell cytotoxicity compared with either agent alone. Notably, while the resistant cell lines AMO-1R180-IXA (IXZ-resistant) and INA-6R17-CAR (CFZ-resistant) retained high viability (~100%) when treated with IXZ or CFZ monotherapy (30 nM), combining these agents with GLUT1 inhibitors (BAY-876 at 2.0 μM or STF-31 at 30 μM) led to a marked and statistically significant reduction in cell viability. In this study, GLUT1 inhibition sensitized multiple myeloma cell lines to proteasome inhibitors and melphalan and overcame acquired carfilzomib resistance. These findings support GLUT1 inhibition as a potential therapeutic strategy to enhance treatment response and address drug resistance in MM.

Poster 23

Deciphering Synaptic Complexity: Tagging Endogenous Neuronal Receptor-Auxiliary Protein Complexes with Genetic Code Expansion (GCE) and Bioorthogonal Click Chemistry

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Most fast excitatory synaptic transmission in the brain is mediated by the AMPA-type glutamate receptors (AMPA-Rs). Over the last years, substantial progress has been made in exploring the molecular composition of AMPAR complexes. A growing number of AMPAR auxiliary proteins have been identified that critically regulate receptor trafficking, synaptic localization, and biophysical properties of these complexes. Despite these advances, the precise composition, nanoscale organization and dynamic behavior of AMPAR complexes in their native cellular environment are not yet fully understood. Addressing these questions requires labeling strategies that provide molecular-level resolution while preserving the ultrastructure and functionality of the receptor complexes. We use click chemistry in combination with genetic code expansion (GCE), which allows bioorthogonal site-specific labeling with minimal linkage error. To achieve labeling of endogenous AMPAR auxiliary proteins, amber mutants are introduced using a CRISPR/Cas9-based Targeted Knock-In with Two (TKIT) guides approach, ensuring precise and effective genome editing. Incorporation of non-canonical amino acids via advanced GCE systems subsequently allows selective conjugation of small fluorescent probes. Ultimately, this strategy enables the visualization and tracking of AMPAR auxiliary proteins in living neurons, with a focus on their spatial distribution and trafficking dynamics during synaptic plasticity processes such as long-term potentiation (LTP) and long-term depression (LTD).

Poster 24

Investigating protein-protein interactions of early spliceosomal assembly protein SF1 variants

Famke Guder, Aganya Basagula Lokesh, Feng Wang, Yongjun Li, Yi Xing, Irene Beusch

A fundamental step of gene expression in eukaryotes is the splicing of pre-mRNA. This process is carried out by the spliceosome, a large molecular complex made up of five snRNAs and hundreds of proteins. During the first steps of spliceosome assembly, the protein splicing factor 1 (SF1) recognizes one of the splicing signals on the pre-mRNA, the intronic branch point sequence, and is thought to help recruit the spliceosome. In humans, SF1 pre-mRNA is extensively alternatively spliced, with two isoforms predominantly expressed across many surveyed cell lines. However, the function of the different SF1 protein variants has to date not been investigated. Sequence differences between the variants locate primarily to the C-terminal end of SF1, which is shown to be involved in protein-protein interactions. It may therefore be assumed that the variants have distinct interactomes and occupy different roles in spliceosome assembly. We have identified mutations in SF1 that link its naturally occurring sequence heterogeneity to the recruitment of the spliceosomal component U2 snRNP to the branch point. This offers an intriguing opportunity to investigate the role of SF1 in alternative splicing and gain insights into early decision making in splicing. Bioluminescence resonance energy transfer (BRET) has been established as a new method in the lab and used to investigate known SF1 interactions, most importantly with U2AF2. Differences in interaction strengths have been observed depending on the interaction partner, the SF1 isoform, the nature of the interaction as well as the position of the luminescent tags. To understand SF1 variant function at the individual level, cell lines expressing single variants, tagged with the biotin ligase ultraID, have been generated. These cell lines will be used in proximity labeling experiments to define the interaction partners of SF1 variants. Induction of gene expression in the cell lines, combined with biotin treatments, resulted in the presence of biotinylated proteins. With these two methods set up, we will be able to investigate the differences in interactomes and potentially functions of different SF1 variants.

Poster 25

NFAT controls T-follicular helper cell (TFH) –TFK cell axis by restricting cytotoxic TFH differentiation

Ali Hassan Warraich, Yin Xiao, Xiaoli Lou, Benjamin Lunz, Friederike Berberich-Siebelt

Recently, we identified a distinct subset of TFH-cells with cytotoxic potential, termed killer TFH (TFK) cells which occur in healthy individuals, but accumulate in the blood of patients undergoing allogeneic HSCT under calcineurin-inhibitor therapy, and those who develop chronic graft-versus-host disease (cGvHD). Since the prime targets of the phosphatase calcineurin are family members of nuclear factor of activated T-cells (NFAT), we investigate the impact of T cell-expressed members NFATc1, c2, and c3, on differentiation of TFK cells. NFAT proteins transmit T-cell receptor signals, i.e. antigen recognition.

In line with calcineurin inhibition in patients, NFAT triple knockout mice with *Nfatc1*^{-/-}*Nfatc2*^{-/-}*Nfatc3*^{-/-} T-cells exhibited an increased number of TFK cells in their spleens and mLN compared to wild-type controls. On contrary, TFH cell frequencies were reduced. Notably, NFATc1 single deficiency mirrored these findings, while NFATc2 or NFATc3 deficiency had minimal effects, suggesting a dominant role for NFATc1. Combined deficiencies further revealed that TFH cells were downregulated and TFK cells expanded in mice with NFATc1c3 double-ablated T-cells and most prominently with NFATc1c2 DKO T-cells. Correspondingly, TFK-to-CD4⁺ cytotoxic T-cell ratio was elevated, indicating enhanced cytotoxic skewing. Interestingly, Peyer's patches (PP) –sites of chronic antigenic stimulation– did not harbor many TFK-cells irrespective of any NFAT deficiency, while *Nfatc1*^{-/-} T-cells still reduced PP-TFH population. Nevertheless, Eomes, one of highly expressed transcription factors in TFK-cells, was still enriched in *Nfatc1*^{-/-} PP-TFH cells, again indicating that NFATc1 prevents cytotoxic TFH cell differentiation.

To further examine TFK development, we co-cultured tonsillar TFH-enriched T-cells with oral-squamous tumor single-cell suspensions. Despite low MHC-II expression, the tumor microenvironment suppressed TFH cells and promoted antigen-independent TFK expansion, potentially via IL-23–driven enhancement of TFK cytotoxic-phenotype. Overall, NFATc1 emerges as a critical regulator of TFH homeostasis, with its deficiency and tumor- or pro-inflammatory conditions favoring TFK expansion. Future studies employing in-vivo cGvHD models essential to elucidate NFAT signaling's role in TFH–TFK balance and TFK function under pathological-condition.

Poster 26

Identification of *Fusarium* spp. within the *Fusarium solani* species complex using MALDI-TOF-MS

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Introduction: *Fusarium* species are clinically relevant molds causing a broad spectrum of diseases ranging from superficial infections in immunocompetent patients to life-threatening invasive and disseminated infections, particularly in immunocompromised patients. Due to their varying susceptibility profiles to antifungals, accurate species identification is essential to guide targeted therapy. Conventional identification methods relying on morphological and microscopic examination are time-consuming, require a high level of expertise and often fail to distinguish closely related species. While molecular methods provide higher accuracy, they are costly and only available in specialized diagnostic laboratories. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has revolutionized routine bacterial and yeast identification and represents a promising alternative for mold identification. However, the discrimination of closely related *Fusarium* species remains challenging due to overlapping protein profiles and a limited number of reference spectra.

Objective: The aim of this study is to evaluate whether MALDI-TOF MS can be used to discriminate closely related species within the *Fusarium solani* species complex using the MALDI Biotyper. **Methods:** A total number of 115 clinical and environmental isolates of the *Fusarium solani* species complex representing 18 different species were provided by the German National Reference Center for Invasive Fungal Infections. Species were identified by sequencing of the translation elongation factor 1-alpha (TEF1 α) and/or the RNA polymerase II second largest subunit (RPB2) genes. Isolates were cultured on Sabouraud Agar for 4-5 days and subsequently transferred to liquid medium. Protein extraction was performed from 18-48 h cultures according to the Filamentous Fungi sample preparation protocol provided by Bruker Daltonics. Mass spectra were acquired using the MALDI Biotyper sirius system (Bruker Daltonics). Spectra underwent quality control and recalibration in FlexAnalysis, followed by generation of reference spectra (MSPs) using Compass Explorer Software. Intra-database comparison of MSPs and visual analysis of spectral peak patterns were performed to evaluate species-specific differences within the *Fusarium solani* species complex.

Results: High quality spectra were obtained for 111 of 115 isolates. Intra-database comparison of MSPs generated from 76 isolates was performed. Discrimination of closely related species was improved, as isolates belonging to the same species and represented by multiple isolates mainly grouped together in the Biotyper ranking lists. Isolates from different species could be separated in the analysis. The simple species-specific overlay of the spectra did not reveal any species-specific masses. To assess diagnostic accuracy of an expanded database for routine use, reevaluation with an independent set of isolates is currently in progress.

Conclusion: These results indicate that MALDI-TOF MS has the potential to discriminate closely related species within the *Fusarium solani* species complex when sufficient numbers of representative reference isolates are included in the database. Expansion of the database may lead to more rapid and reliable species-level identification in routine diagnostics and thereby support fast and targeted antifungal therapy.

Poster 27

Conserved but Distinct: Capsid Structural Variations Across the Mammalian Orthohepadnavirus Genus

Naomi Hemmelmann

Hepatitis B Virus (HBV) is a known human pathogen. Infecting over 250 million people worldwide, it is a major health concern often leading to chronic infection in carriers and consequent cirrhosis of the liver or liver cancer. While human HBV has been extensively studied, comparatively little is known about the structural biology of the broader Orthohepadnavirus genus infecting other mammals. Based on sequence conservation, these viruses are predicted to be structurally highly similar. This has been confirmed by the structural characterization of the Woodchuck Hepatitis B Virus (WHBV) capsid. However, preliminary investigation into the structure of other members of the Orthohepadnaviridae family suggests that even within this highly conserved genus, small but noticeable differences exist. Residues associated with key functions within the life cycle of human HBV, such as envelopment and secretion, are not conserved in other members of the genus. Similarly, variations have been observed on a structural level concerning the intra-dimer packing within the spike. While these differences are not as pronounced as those between genera, they nonetheless suggest an adaptation of the viral capsid to its specific host, even within the same genus, and could provide insight into the viral capsid's involvement in host tropism.

Poster 28

GPVI haplotypes have no prognostic impact in adult sepsis

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Background: Sepsis is a life-threatening condition consisting of a dysregulated immune reaction during an infection resulting in multiple organ failure. Despite thrombocytopenia and bleeding during sepsis, thrombotic events occur. Our laboratory identified a reduced platelet reactivity upon stimulation of the collagen receptor GPVI, as an indicator of early sepsis and a possible approach to a mechanistic understanding and treatment of the observed platelet dysfunction. Several GPVI haplotypes have been characterized, which differ in a series of distinct single nucleotide polymorphisms (SNPs). Within these, the aa (68%) and ab (24%) haplotypes are the most common, while the bb (and other rare) haplotypes account for only 8% of individuals. In a paediatric sepsis cohort, Asfari et al. have reported different organ dysfunction scores (PRISM III and PELOD-2) between GPVI subgroups stratified by haplotype (aa (GPVIa), ab (GPVIb)). However, its impact on adult sepsis is unknown. Here, we aimed to evaluate the impact of GPVI haplotypes on platelet hyporeactivity, disease severity and clinical outcome in an adult patient with sepsis.

Methods: We recruited 111 patients with sepsis and 52 patients with infection at the University Hospital Würzburg as well as 29 healthy controls (EV234/21, EV102/17 and EV94/19). GPVI aa and ab haplotypes were determined by Sanger sequencing the relevant sequences in exon 7+8 of the GP6 gene. Demographic and clinical data were collected; the GPVI expression and platelet reactivity were assessed by flow cytometry.

Results: We confirmed that platelets in sepsis show reduced GPVI expression and are hyporeactive towards the GPVI agonist CRP-XL (collagen-related peptide). In healthy controls, the bb haplotype shows a markedly decreased GPVI expression and reactivity compared to aa and ab. In contrast to the paediatric cohort, we did not detect a difference in organ dysfunction (SOFA) score or clinical characteristics between GPVI haplotypes aa and ab in our adult sepsis cohort, nor a functional difference in vitro. In our infection cohort, the bb haplotype was, unexpectedly, overrepresented.

Conclusion: We demonstrate that in contrast to paediatric sepsis GPVI haplotypes have no impact on disease severity in adults. This underlines that sepsis in adults and children, especially neonates, represent different disease conditions, most probably due to differences in immune response, platelet function and coagulation.

Poster 29

Preconditioning of Monocytes with MF59 Promotes Enhanced Differentiation and Activation of Dendritic Cells

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The oil-in-water emulsion MF59 is a widely used vaccine adjuvant known to enhance immune responses, yet its direct effects on dendritic cell (DC) differentiation remain incompletely understood. Therefore, in this exploratory experimental study, we investigated the impact of MF59 on the in vitro differentiation of DCs from human monocytes (n = 3 donors).

CD14-sorted peripheral monocytes were preconditioned with MF59, followed by a resting phase in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-4 (IL-4) to induce monocyte differentiation into immature dendritic cells (iDCs). Expression of the differentiation and activation markers CD80, CD83, CD86, CD40 and HLA-DR was determined by flow cytometry.

Phenotypic analysis revealed that MF59 preconditioning increased the expression of CD86 and HLA-DR on iDCs by a mean of 46.7% and 16.3%, respectively, compared to iDCs induced from GM-CSF/IL-4-treated controls, while no differences in the expression of CD80, CD83, and CD40 were observed. Given the small sample size (n = 3), no statistical testing was performed. Results should be regarded as descriptive trends. Elevated expression of CD86 and HLA-DR (22.2% and 12.0% higher than controls, respectively) was preserved after differentiation into mature DCs (mDCs).

To further elucidate the underlying mechanisms and functional consequences of MF59 preconditioning, co-culture experiments with autologous T cells and influenza antigen challenge, together with cytokine profiling and RNA sequencing analyses, are currently being performed to characterize antigen-specific T cell activation and the molecular and transcriptional changes associated with MF59 exposure. These findings suggest that MF59 not only acts as an adjuvant at the level of immune activation but may also modulate early monocyte-to-DC differentiation, promoting a more immunostimulatory phenotype.

Poster 30

Effects of translocating vancomycin-resistant *Enterococcus faecium* and their derived membrane vesicles on intestinal barrier integrity

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Background

Enterococcus faecium is intestinal microbiota that can cause life-threatening conditions in case of the disruption of the gut barrier integrity ("leaky gut"). This enables translocation of enterococci into the blood stream, and therefore harbours the risk for critical infections in hospitalized, debilitated patients. Still, the underlying mechanisms are not completely understood. Another important factor in pathogenesis is bacterial membrane vesicles (MVs), as they are involved in various biological processes, such as the secretion of virulence factors or host-pathogen interactions. However, their potential influence on translocation of vancomycin-resistant *E. faecium* (VREfm) has not been determined.

This study aims to target the molecular mechanisms of intestinal translocation of different VREfm subtypes and their MVs in a healthy and a disrupted gut barrier model, by investigating their translocation potential as well as the bacterial influence on barrier integrity.

Methods

A differentiated Caco-2 based transwell model was used to investigate the translocation potential of VREfm and their derived MVs. To mimic a disrupted gut barrier, differentiated desmoglein-2- deficient Caco-2 (Dsg2-/-) monolayers were used. As an additional impaired model, differentiated Caco-2 monolayers were incubated with a mixture of different cytokines ("cytomix") for 24 h. For investigations of barrier stabilizing effects of sitagliptin, Caco-2 monolayers were co-incubated with 1 μ M sitagliptin and cytomix with a pre-incubation of sitagliptin for 2 h. To investigate bacterial translocation, monolayers were treated with either VREfm or their derived MVs for 6 h. Transepithelial electrical resistance (TEER) measurements were performed during 6 h of infection to examine the bacterial influence on barrier integrity.

Results

VREfm were able to translocate across intact and impaired monolayers within 6 h of infection. Impaired models showed a slight increase in bacterial translocation compared to the healthy model. TEER measurements indicated a general increase in TEER within 6 h of infection, with a significant increase ($p < 0.02$) in infected monolayers treated with cytomix or co-incubation. Incubation with bacterial MVs revealed a significant decrease in TEER in healthy barriers ($p = 0.049$) and Dsg2-/- monolayers ($p = 0.036$) after 6 h of infection. Impaired monolayers incubated with MVs showed a stronger decrease in TEER compared to healthy monolayers.

Conclusion

Our results suggest that VREfm are not only able to translocate across healthy and impaired intestinal barriers, but that their derived membrane vesicles could also play a crucial part in disrupting the intestinal barrier for following translocation events. Further investigations are needed to verify the role of MVs in bacterial pathogenesis, including intestinal translocation and barrier weakening functions.

Poster 31

A multiparameter whole blood platform for assessment of anti-CMV immunity in allogeneic stem cell transplantation recipients

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Despite prophylaxis, clinically significant cytomegalovirus infection (csCMVi) remains a major cause of morbidity and mortality after allogeneic stem cell transplantation (alloSCT)^{1,2}. Current immune monitoring assays are IFN- γ -focused, time-consuming, vulnerable to pre-analytical variation, and lack sensitivity and specificity^{3,4}. We therefore established a multiparameter whole blood assay that can be stimulated directly at the bedside, preserves a more physiological immune cell environment, and may provide a more comprehensive assessment of HCMV-specific immunity through the inclusion of diverse immune parameters. Whole blood and/or PBMCs from 39 adult alloSCT recipients were analyzed. V δ 1⁺ $\gamma\delta$ T cells, memory-like NK cells, and HCMV-specific memory B cells were quantified by multicolor flow cytometry. HCMV-specific T cells were measured either directly ex vivo using HLA class I multimers (CD8⁺) or following 20–22 h stimulation with HCMV peptides (CD8⁺ and CD4⁺) under two co-stimulatory conditions (α -CD28/ α -CD49d/Brefeldin A vs. α -CD28/ α -CD40). Multiplex cytokine analysis was performed in PBMC supernatants or plasma following AIM stimulation. Finally, the feasibility of analyzing these complementary immune parameters in parallel on the following day within a single integrated workflow was assessed. V δ 1⁺ $\gamma\delta$ T cells, memory-like NK cells, HCMV-specific memory B cells, and HLA class I multimer-positive T cells were detectable in WB from HCMV-seropositive patients, with specificity confirmed in seronegative controls. AIM responses did not differ between the two co-stimulatory conditions, allowing omission of Brefeldin A and enabling parallel cytokine quantification. WB and PBMCs showed comparable HCMV-specific responses, while WB yielded significantly higher frequencies of activated CD8⁺ T cells in response to the HCMV peptide pool (7.9% vs. 1.5%; $p=0.007$) in AIM assays. CD4⁺ responses were similar between the two systems (0.5% vs. 0.4%; $p=0.08$). CMV-specific T-cell responses correlated between WB and PBMCs for both CD8⁺ ($p=0.0238$) and CD4⁺ ($p=0.0187$) responses. All investigated immune parameters could be analyzed in parallel within a single-day workflow. In a pilot cohort of 12 patients, this integrated approach enabled identification of patients who subsequently developed virus-related events, highlighting its potential clinical relevance. The WB-based assay enables sensitive detection of key HCMV-protective immune populations and reduces pre-analytical variability by allowing bedside stimulation. Future studies should analyze which single or combination of immune cell parameters are predictive for csCMVi and evaluate its predictive value against the current clinical standard, PBMC-based ELISpot.

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Poster 32

Transcriptomic Analysis of T-cell in Acute Myeloid and Acute Lymphoblastic Leukemia reveals Altered Apoptosis

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T-cell dysfunction is an important feature of the leukemic bone marrow microenvironment. In this study, we investigated transcriptional changes in T cells from patients with acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) compared with healthy donor T cells. Single-cell RNA-seq data from the GSE154109 dataset were analyzed using differential gene expression and GO and KEGG pathway enrichment analyses.

AML- and ALL-associated T cells showed several common transcriptional changes. In both diseases, upregulated genes were enriched in RNA processing and RNA splicing, whereas downregulated genes were associated with T-cell activation and T-cell receptor (TCR) signaling. AML and ALL shared 289 upregulated and 211 downregulated genes, with several shared genes showing stronger expression changes in AML.

Apoptosis-related pathways were enriched in both AML- and ALL-associated T cells, representing another shared alteration between the two leukemia types. Despite these similarities, some differences were observed in the pathway analysis. ALL showed additional enrichment of TNF signaling and T-cell differentiation pathways, whereas IgSF CAM signaling was enriched among the downregulated genes in AML.

TMEM204 was consistently downregulated in T cells from both AML and ALL compared with healthy controls. TMEM204 is a multi-pass transmembrane protein reported to localize to the plasma membrane and to be associated with cell adhesion, adherens junctions and cellular permeability. Although its membrane localization makes it an interesting candidate in the context of the altered T-cell phenotype observed in our analysis, there is currently insufficient evidence to consider TMEM204 a specific T-cell surface marker or to conclude that it directly regulates TCR signaling. Its consistent downregulation in both leukemia types therefore warrants further investigation.

Overall, AML and ALL show common alterations in RNA processing and T-cell signaling together with disease-specific differences. The apoptosis-related changes and the consistent downregulation of TMEM204 provide interesting directions for further investigation of T-cell dysfunction in leukemia and potential therapeutic targets.

Poster 33

Cell-type-specific proteostasis alterations in Alzheimer's disease mice

Emma Karp, Marco Niestroj, Marta Zaninello, Irina Dudanova

Neurodegenerative diseases are characterized by progressive loss of neuronal function that fundamentally disrupts brain homeostasis, often driven by impairments of the cellular proteostasis network and accumulation of misfolded protein aggregates. The proteostasis network, comprising tightly regulated processes mediated by molecular chaperones and degradation pathways, maintains protein homeostasis but undergoes progressive and selective decline with aging and genetic risk, rendering certain neural cell types vulnerable to toxic aggregate accumulation. A hallmark of neurodegenerative diseases is the selective vulnerability of distinct neuronal subtypes, yet the molecular determinants underlying this differential susceptibility remain poorly understood. The two main types of protein pathology in Alzheimer's disease are extracellular A-beta-plaques and intracellular tau neurofibrillary tangles, which exert toxicity on neurons by distinct mechanisms. The main goal of this project is to elucidate how different populations of cortical neurons are selectively affected by tau and/or A-beta pathology and whether this is driven by defects in proteostasis. Building on our previously established Fluc-EGFP proteostasis reporter mouse line, we are conducting histological analyses of the neocortex in tauopathy and APP mouse models at different time points during disease progression. We investigate glutamatergic projection neurons and several types of GABAergic interneurons, including parvalbumin, somatostatin and VIP cells. By directly comparing proteostasis network components and aggregate burden across specific neuronal types, the study aims to identify key regulatory mechanisms and actionable therapeutic targets for mitigating proteostatic failure in neurodegenerative diseases.

Poster 34

Mechanically Optimizing Cytotoxic T Lymphocytes to Combat Soft Circulating Tumour Cells

Garima Kashyap

T cells are constantly scanning the environment and perceiving cues which impact their behavior. In effector CD8⁺ T cells, also known as cytotoxic T lymphocytes (CTLs), substrate and target stiffness regulate diverse biological functions, including migration, synapse organization, degranulation, and serial killing. These observations suggest that CTLs sense the mechanical properties of their surrounding microenvironment, allowing their responses to be shaped by substrate stiffness. However, metastatic tumour cells may exploit the functional trait of stiffness as a potential mechanism of immune evasion. Cancer cells have been shown to be significantly softer than their healthy counterparts, and this biophysical transformation correlates with increased metastatic potential. We know that T cells actively sense physical cues and follow stiffness gradients for their migration - a process called durotaxis. Their migration and the subsequent activation rely on a delicate balance between actin-myosin contractility and integrin-based adhesion—essentially a molecular "clutch and motor" system.

However, no study has yet systematically re-engineered this internal machinery of the effector CD8⁺ T cells to retune their mechanosensitivity, specifically toward the soft environment, which is a characteristic of metastatic cells.

This project aims to bridge this gap by using CRISPR-Cas9 to modify key actin-myosin and integrin-clutch components, including ITGA1, ITGA2, ITGAV, MYO1B, MYO2B, and MYO9B, in CTLs. By shifting their optimal mechanical response toward softer environment, we aim to characterize how the CTLs migrate and activate across varying levels of stiffness. Ultimately, this work seeks to establish design principles for CAR T-cell therapies to target a new kind of metastatic marker: the altered cytoskeletal structure that may serve as a mechanical hallmark of metastatic cancer cells.

Poster 35

Actin conformation dynamics precede force generation in cytotoxic T lymphocytes

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Cytotoxic T lymphocytes (CTLs) are cells of the adaptive immune system that are able to recognise and kill cancerous or virally infected target cells. The biochemical basis of CTL-mediated killing has been examined in great detail. Recent work has underscored the importance of physical forces in T cell function too, but how CTLs sense and exert force during migration and killing is not fully understood. Here, by expressing actin conformation probes based on the CH domain of utrophin, we directly visualize regions of altered F-actin conformation in primary CTLs during migration and killing. By combining these probes with traction force microscopy, we correlate external force with the internal cytoskeleton. We find strong, forward-directed traction forces at the leading edge of migrating CTLs that correlate with regions dominated by a lamellipodium-enriched actin conformation, and show that inhibition of non-muscle myosin IIa, Arp2/3, or formins disrupts force production without disrupting this biased actin conformation, indicating that conformation is regulated upstream of force. At the cytotoxic immune synapse, force exertion peaks 50-100 seconds after an early, force-associated actin conformation dominates the synapse, with this pattern conserved in physiological CTL-target cell conjugates. We show that actin conformation is regulated upstream of force production, and that force exertion at the cytotoxic immune synapse temporally follows actin conformation dynamics. Our work offers insight into the relationship between cellular force production and F-actin conformation in important primary immune cells.

Poster 36

Characterization of novel multispecific nanobody fusion proteins targeting CD70

Maximilian Kempf, Isabell Lang, Harald Wajant

CD27 is a costimulatory receptor of the Tumor necrosis factor (TNF) super family (TNFRSF), expressed mostly in naïve and memory T cells and is activated by its only known ligand, CD70 (=CD27L). At the cell membrane, CD27 can form pre-assembled dimers and higher-order receptor clusters. Upon engagement by trimeric CD70 expressed on the surface of neighboring cells, CD27 clustering is promoted, thereby initiating intracellular signaling in the CD27-expressing cell and activating both canonical and non-canonical NF- κ B pathways. Under physiological conditions, CD70 is only transiently expressed by specific cell types like activated dendritic cells, B cells, and T cells. However, in some tumors, CD70 is constitutively expressed, leading to T cell exhaustion and immune evasion. Therefore, targeting the CD27/CD70 axis is being strongly investigated.

In my master's thesis, I characterized multivalent nanobody-based CD70 targeting constructs in cellular and non-cellular binding studies, ELISA analyses and reporter assays. After evaluating the binding affinity of different CD70 nanobodies, their ability as antagonists of CD27/CD70-driven activation of CD27-expressing cells was tested in ELISA using Fc-fused constructs. For further investigation, bispecific and trispecific nanobody-based fusion proteins were generated and analyzed for their capacity to exploit CD70-dependent cell surface anchoring to enhance the agonistic activity of nanobodies targeting other members of the TNFRSF. For this purpose, the constructs were tested in coculture ELISA assays using CD70-expressing cells as anchoring cells and TNFRSF-responsive target cells as reporter cells.

This assay design is based on the principle that efficient activation of many signaling-competent TNFRSF receptors critically depends on the formation of signaling-competent higher-order receptor clusters. Whereas membrane-bound TNFSF ligands promote such clustering at cell–cell contact sites, soluble or monovalent receptor-binding molecules generally display no or only limited agonistic activity.

In line with this model, our data indicate that incorporation of a CD70-specific nanobody module into bispecific or trispecific constructs enhances the agonistic activity of the receptor-targeting nanobody in the presence of CD70-expressing cells. In this format, the CD70-specific nanobody functions as a cell surface anchoring module, thereby locally enriching the fusion protein at CD70-positive cells. Given the constitutive expression of CD70 in several tumors, this strategy may help to selectively concentrate agonistic TNFRSF-targeting fusion proteins within CD70-positive tumor microenvironments.

Poster 37

Molecular and Cell Biological Investigations of Echinococcus Stathmin as a Regulator of the Cell Cycle

Karim Kessler

Background: *Echinococcus multilocularis* is the causative agent of alveolar echinococcosis (AE), a potentially fatal zoonotic disease often requiring extensive surgery and long-term parasitostatic chemotherapy [1,2]. Germinative cells constitute the proliferative stem cell population of the parasite and are therefore of particular interest as potential therapeutic targets [3]. Furthermore, alterations in parasite tubulin have been associated with benzimidazole resistance, with the strongly expressed Tub-2 isoform exhibiting reduced benzimidazole sensitivity in germinative cells and potentially contributing to the limited efficacy of treatment against this cell population [3,4]. **Objectives:** This study aimed to characterize *E. multilocularis* Stathmin (EmuJ_000929400) as a potential germinative-cell marker and investigate its association with tubulin-related cellular processes. Additionally, the effects of selected benzimidazole compounds on parasite larval stages and germinative-cell proliferation were investigated.

Methods & Results: Bioinformatic analyses revealed high sequence homology of *E. multilocularis* Stathmin with Stathmin homologues from other cestodes, including *Mesocostoides corti* and *Taenia asiatica*, while distinct sequence differences were observed compared with human Stathmin. Predicted tertiary protein structures were highly conserved across cestode and human homologues. Whole-mount in situ hybridization demonstrated prominent Stathmin expression in germinative cells of *E. multilocularis* metacestodes, with expression associated with the cell cycle. Overexpression of *E. multilocularis* Stathmin in HEK cells did not reproduce previously described inhibitory effects on cellular branching [5]. Yeast two-hybrid experiments did not detect an interaction between Stathmin and the Stathmin-regulating kinase Cdk1. In vitro treatment with mebendazole, cambendazole, febantel and fenbendazole over 14 days resulted in pronounced reductions in germinative-cell proliferation. EdU detection was markedly reduced following treatment with 10 and 30 μ M fenbendazole and 30 μ M febantel. Fenbendazole additionally appeared to affect differentiated parasite tissues, including the laminated layer and tegument.

Conclusion: *E. multilocularis* Stathmin shows strong germinative-cell-associated and cell-cycle-dependent expression, supporting its potential as a marker of the proliferative parasite stem cell population, while its precise functional role remains unclear. The pronounced effects of fenbendazole and febantel on germinative-cell proliferation are particularly promising, as benzimidazoles have so far shown limited efficacy against this proliferative stem cell population. These findings may therefore provide an important basis for the development and optimization of therapeutic strategies targeting *E. multilocularis* germinative cells.

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Poster 38

RHODOPSIN 7 influences behavior during changes in light intensity in *Drosophila melanogaster*

Valentina Kirsch, Amelie Brönnert, Nina Stenger, Charlotte Helfrich-Förster, Pingkalai R. Senthilan

Light perception regulates diverse biological processes beyond image formation, including circadian rhythms and behavioral adaptation. In *Drosophila melanogaster*, rhodopsins are light-sensitive G protein-coupled receptors that convert light into intracellular signals. Among the seven *Drosophila* rhodopsins, RHODOPSIN 7 (RH7) is unique in its structure and expression pattern and has a non-image-forming role.

To investigate its role in light-dependent behavior, we analyzed Rh7⁰ mutants and observed altered responses to light transitions, including changes in startle and optomotor responses as well as phototaxis.

We also examined Darkfly, a strain maintained in complete darkness for more than 1400 generations that accumulated multiple mutations, including a nonsense mutation in Rh7 resulting in the loss of the final 21 amino acids. To test the functional relevance of the Darkfly Rh7 variant independently of additional mutations, we generated a Rh7^{Darkfly} CRISPR/Cas9 line expressing the truncated RH7 protein and analyzed its behavioral responses.

Overall, our results provide further evidence that RH7 contributes to behavioral responses to changes in light intensity and offer new insights into the function of this unusual rhodopsin in *Drosophila melanogaster*.

Poster 39

Analysis of spinal neuron populations and their descending inputs in mouse models of Huntington's disease

Sarah Kläffgen, Ebru Ozer Yildiz, Ivana Jeremic, Miguel da Silva Padilha, Ines Klein, Graziana Gatto, Irina Dudanova

Huntington's disease (HD) is a devastating neurodegenerative disorder caused by an expansion of CAG repeats in exon 1 of the Huntingtin gene. This mutation results in the misfolding and dysfunction of the huntingtin protein, which accumulates in intracellular inclusion bodies throughout the brain. HD particularly affects brain regions involved in motor control, and the role of the neocortex and basal ganglia in disease pathogenesis has been studied extensively. However, little is known about how distinct spinal neuronal populations are affected and how remodeling of spinal motor circuits during disease contributes to motor defects. In this project, we aim to identify the spinal neuronal populations and their supraspinal afferents that are selectively affected in HD and might cause distinct changes in motor execution. To this end, we use a combination of histological and behavioral analyses in two mouse models of HD at different disease stages: the slowly progressing zQ175DN knock-in model that is genetically similar to the human patients and the rapidly progressing R6/2 model that has strong disease phenotypes.

Our preliminary data reveal a distinct gait pattern in 12-week-old R6/2 mice, which lift their ankles higher during the swing phase of locomotion compared to wild-type controls. Furthermore, we detected the accumulation of inclusion bodies and diffuse mutant huntingtin throughout the spinal cord of 8-week-old (around disease onset) and 12-week-old (advanced stage) R6/2 mice. In advanced stage zQ175DN mice (15–17 months old), we observed an increased density of both inhibitory and excitatory spinal interneurons. Furthermore, we found a reduction in ChAT-positive cells in the cervical spinal cord, whereas no such reduction was detected in the lumbar spinal cord. Taken together, these data suggest HD causes morphological and molecular changes in distinct neuronal cell types in the spinal cord, and paves the way for a detailed investigation of the role of spinal cord circuits in this movement disorder.

Poster 40

Towards prediction of antibiotic Mode of Action using high-throughput imaging and AI

Felix Knotz, Christophe Zimmer

Antibiotic resistance is fueling a growing global health crisis, necessitating the discovery of antibacterial compounds with novel modes of action (MoAs). The decline in new antibiotic approvals, coupled with the rise of multi-resistant pathogens, highlights the limitations of traditional antibiotic drug discovery. Among them, phenotypic drug screening has proven effective at identifying antibacterial compounds, but requires time-consuming follow-up experiments to determine drug MoA or targets.

Here, we will outline a project aiming to develop a streamlined workflow for predicting antibiotic MoAs from automated microscopy imaging data analyzed by deep learning. The project is based on the hypothesis that MoAs of chemical compounds can be predicted by computationally matching their induced phenotypes to those of genetic mutants. In order to map the phenotypes of essential genes, we use a CRISPRi technique allowing to induce their repression in a finely tuned manner. We will report on our early efforts to create and phenotype these inducible mutants in *E. coli*. This work contributes to the establishment of a pipeline that will accelerate the search for novel antibiotics against antimicrobial resistant bacteria.

Poster 41

Targeting the R95 Pocket of the AAA+ ATPase p97: A Crude-Reaction-Mixture and Virtual-Screening Platform for Fragment-to-Lead Optimization

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Background: p97 (VCP/Cdc48) is an essential AAA+ ATPase whose hexameric D1/D2 domains drive numerous proteostatic processes, including ERAD, chromatin-associated degradation and autophagy. ATP hydrolysis in the D1 ring is coupled to the position of the N-terminal domain, which toggles between a “down” (ADP-like) and an “up” (ATP-like) conformation. Disease-associated substitutions at the N-D1 interface and linker — R95G, R155H, A232E and L198W — shift this equilibrium toward the up/ATP-like state even when ADP is bound (Schuetz & Kay, eLife 2016, 5:e20143), deregulating ATPase activity and downstream cofactor recruitment. We aim to identify small-molecule modulators that bind the R95 pocket at this interface and restore the ADP-like conformation, providing both a potential therapeutic strategy and a chemical probe for p97 conformational dynamics. **Methods:** Three independent fragment screening campaigns — 19F NMR, Grating-Coupled Interferometry (GCI) and X-ray crystallography — were carried out against p97 ND1. Only the X-ray screen yielded hits occupying the R95 pocket; these were progressed by classical medicinal chemistry — analog synthesis and bioisosteric replacement addressing a pocket lysine residue and defining growth vectors — with binding orthogonally validated by STD-NMR and confirmed by X-ray co-crystallography and GCI. To explore chemical space with limited synthetic resources, we adopted a crude-reaction-mixture (CRM) strategy analogous to the Binding-Site Purification of Actives (B-SPA) approach (Grosjean et al., Angew. Chem. Int. Ed. 2025): fast, optimized reactions (amide coupling/ester hydrolysis) were run in array format and screened directly, without purification, by X-ray crystallography and GCI. An in-house solid-phase synthesis platform is being established in parallel to further expand accessible chemical space for this and other projects.

Results: In parallel, a 19F NMR fragment screen (~190–230 compounds) against p97 ND1 WT gave a hit rate of ~5%, and an independent GCI fragment screen was performed; neither screen targeted the R95 pocket specifically. X-ray fragment screening yielded multiple hits in the R95 pocket; subsequent medicinal chemistry produced analogs addressing the pocket lysine and defined promising exit vectors, with binding confirmed both structurally (X-ray) and in solution (STD-NMR, GCI). Reaction conditions for the CRM array chemistry have been optimized, and ~100 compounds have been synthesized and screened to date, establishing a resource-efficient pipeline for fragment growing in a small team. To prioritize the next synthesis round, an in silico library of ~500,000 building blocks is currently being triaged by virtual screening (SeeSAR, HYDE scoring/FlexX docking), with prioritized compounds to be synthesized and validated by X-ray crystallography and GCI.

Poster 42

Human-targeted drugs extensively reshape regulatory networks controlling virulence and antibiotic resistance in *Salmonella Typhimurium*

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Human-targeted drugs are increasingly recognized to affect bacterial physiology, yet their impact beyond growth inhibition, and the underlying molecular mechanisms, remain poorly understood. Here, we systematically mapped how chemically diverse host-associated compounds influence virulence and intrinsic antibiotic resistance in *Salmonella enterica* serovar Typhimurium. Using a high-throughput reporter approach, we quantified transcriptional response of 26 key stress promoters in *Salmonella* against 2,415 compounds, including FDA-approved drugs, food-derived molecules, and human metabolites. We found that non-antibiotic compounds induce transcriptional responses distinct from those elicited by antibiotics, uncovering a regulatory shift from general stress responses to compound-specific regulation driven by ligand-responsive MarR- and TetR-family regulators. In particular, more than 200 non-antibiotic drugs modulate virulence or intrinsic resistance pathways through the central regulators SlyA or RamR, respectively. By integrating our large-scale dataset with machine learning, we further defined chemical features associated with ramA transcriptional activation in *Salmonella*, and predicted ~200 additional candidate inducers of intrinsic antibiotic resistance in this pathogen. Together, our results uncovered a large and previously unrecognized chemical landscape that reshapes bacterial stress responses through ligand-responsive regulatory mechanisms, with implications for how host-associated compounds influence pathogen virulence and antibiotic resistance.

Poster 43

Modification of the auxiliary subunit $\alpha 2\delta 2$ (Cacna2d2) of the Cav2.2 calcium channel and its influence on the maturation and function of motoneurons in a mouse model of spinal muscular atrophy (SMA)

David Kranich, Ezgi Yildirim, Natascha Schäfer, Sibylle Jablonka

Spinal muscular atrophy (SMA) is a severe childhood neuromuscular disorder caused by the loss of the Survival Motor Neuron (SMN) gene. Although disease-modifying gene therapies have significantly improved patient outcomes, the cellular mechanisms underlying impaired neuromuscular junction (NMJ) function remain incompletely understood. Presynaptic calcium dysregulation is considered a key contributor to NMJ dysfunction in SMA. Active zone-associated voltage-gated calcium channels (Cav2.2) are essential for calcium influx and synaptic vesicle release at motor neuron presynapses. Their trafficking modulation, membrane localization and clustering is influenced by the auxiliary subunit $\alpha 2\delta 2$ (Cacna2d2). Alterations in $\alpha 2\delta 2$ expression are therefore expected to directly affect calcium channel abundance and function, influencing neuronal excitability and synaptic transmission. The aim of this thesis is to investigate the role of Cacna2d2 in calcium signaling and Cav2.2 channel regulation in the context of SMA. Specifically, we will examine how Cacna2d2 knockdown affects spontaneous calcium transients, Cav2.2 channel cluster formations and channel expression levels in Smn-deficient mouse motoneurons. As a control, we will investigate the effects of Cacna2d2 overexpression in wild type motoneurons. To assess morphological changes, Cav2.2 channel signal intensity, growth cone size, and axon length will be quantified. Functional changes will be evaluated by imaging of spontaneous calcium influx. Elucidating the functional relationship between Cacna2d2 and Cav2.2 in Smn-deficient motoneurons will improve our understanding of cellular differentiation and neurotransmission and may point out additional potential therapeutic targets for restoring neuromuscular transmission in SMA.

Poster 44

Scalable Data-Driven Discovery of Imine Photoswitches Through Property-Driven Multi-Objective Optimization

Lasse Kreimendahl, Jiarong Wu, Jake L Greenfield, Roland Mitric

Molecular photoswitches based on E/Z isomerization are central to the development of light-responsive materials, molecular machines, and dynamic-covalent systems. While azobenzene derivatives dominate this field, their imine analogues have historically been overlooked due to poor thermal stability, incomplete switching, and reliance on UV excitation. Recent work has demonstrated that aryliminopyrazoles constitute a promising class of imine photoswitches, combining quantitative visible-light switching with long-lived metastable states and high synthetic accessibility. These advances motivate a systematic expansion beyond the limited set of experimentally explored motifs. Here, we present a computational framework for the large-scale discovery of imine-based photoswitches. Building on established structure–property relationships and a validated quantum chemical protocol capable of reliably predicting thermodynamic and photophysical properties, we construct a hypothetical design space comprising over 25 million imines. This space is generated via combinatorial coupling of chemically realistic amine and aldehyde fragments, ensuring synthetic plausibility while enabling extensive chemical diversity.

To efficiently navigate this vast space, we employ Bayesian optimization coupled to our quantum chemical workflow. This approach enables the targeted identification of candidate photoswitches that optimize multiple objectives, including high thermal stability of the metastable Z-isomer and selective activation with visible light. Furthermore, we outline strategies for experimental validation and iterative model refinement, enabling feedback between computation and synthesis.

Poster 45

Understanding the modulation of the DNA repair enzyme hOGG1 under oxidative stress

Anil Kumar, Pia T Foehrenbacher, Omkar RajendraValanju, Hans Michael Maric, Ingrid Tessmer

Reactive oxygen species (ROS) lead to the formation of highly mutagenic 8-oxoguanine (oxoG) lesions in DNA. The human DNA repair enzyme oxoguanine glycosylase 1 (hOGG1) recognizes and excises mutagenic oxoG lesions and initiates the base excision repair (BER) pathway for their repair. In addition to its canonical function in BER, hOGG1 has been shown to regulate the expression of inflammatory genes under oxidative stress by recruiting transcription factors such as the Myc oncogene to gene promoters. Recent findings from our lab¹ showed direct interaction between hOGG1 and Myc, which is enhanced under oxidative stress and promotes Myc loading at E-boxes within promoters and thus inflammatory gene activation and tumor progression. The development of inhibitors to block the hOGG1-Myc interaction would thus offer high potential for tumor therapy. Our studies also revealed that hOGG1 dimerization induced by oxidative conditions is responsible for the enhanced hOGG1-Myc interaction *in vitro*. Here, we demonstrate hOGG1 dimerization under oxidative stress *in vivo*, and structurally and functionally characterize the “*in vivo* dimer”. Single-molecule atomic force microscopy (AFM) and fluorescence-coupled optical tweezers (CTrap) as well as biochemical assays demonstrate that the “*in vivo* dimer” retains DNA-binding affinity, lesion recognition specificity, and glycosylase activity. We finally use cryo-EM to characterize the structural properties of the hOGG1 dimer and ultimately the hOGG1-Myc binding interface to understand its regulatory mechanism under oxidative stress towards targeted inhibitors for cancer therapy.

Poster 46

Probing the cellular target engagement of phosphoglycolate phosphatase inhibitors with NanoBRET assays

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Phosphoglycolate phosphatase (PGP) is a metabolic phosphatase of the haloacid dehalogenase (HAD) superfamily. By dephosphorylating glycolysis-associated, erroneous metabolites, PGP activity is critical for maintaining high flux through glycolysis and the pentose phosphate pathway under conditions of increased metabolic demand. Targeting cellular glucose metabolism is a promising therapeutic strategy in oncology, which we aim to exploit through the development of novel PGP inhibitors.

Since the discovery of the first PGP inhibitor, CP1, structure-activity relationship (SAR) optimization has led to the identification of increasingly potent and selective PGP inhibitors. However, a critical gap remains in demonstrating direct target engagement and intracellular binding kinetics, limiting the mechanistic characterization of inhibitors and validation of PGP as a therapeutic target. To address this, we aim to establish a NanoBRET-based assay for the direct and quantitative measurement of PGP target engagement in living cells. We present a panel of NanoBRET probes designed to preserve PGP binding affinity while positioning the fluorophore outside the active site to maximize BRET efficiency. All tracers were evaluated in Nluc-PGP and Nluc-PDXP cells to assess target specificity. The pyridoxal phosphatase PDXP is closely related to PGP and serves as a phosphatase control. Based on their PGP-specific target engagement, the most promising tracers were subsequently selected to characterize the cellular target engagement of novel PGP inhibitors. This NanoBRET assay provides a valuable tool for the characterization and validation of novel PGP inhibitors in a cellular context, enabling insights into their cellular activity and therapeutic potential.

Poster 47

Uncovering Phenotypic Heterogeneity of Intracellular Bacterial Subpopulations

Hao Yang Lay, Camilla Ciolli Mattioli

It is known that bacterial cells within a genetically identical population can exhibit phenotypic heterogeneity. In this project, by utilizing fluorescent-based reporter systems and FACS, we will enrich for specific bacterial subpopulations based on known markers for bacterial metabolic states within an infection context. We then utilize Microcolony-seq, a methodology that captures inherited heterogeneity of single bacterial cells, coupled with downstream transcriptomic analyses and functional assays. This will allow us to uncover the transcriptional heterogeneity within bacterial subpopulations based on known markers for bacterial metabolic states.

Poster 48

PP1B is a novel regulator of DNA damage response and cell-cycle regulation during megakaryocyte development

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Background

Platelets are anucleate cells derived from megakaryocytes (MKs) and are essential for hemostasis. Despite their critical physiological and clinical importance, the molecular mechanisms governing MK maturation, polyploidization, and platelet production remain incompletely understood. Phosphorylation and dephosphorylation are key regulators of MK development and thrombopoiesis. Although protein kinases have been extensively investigated in these processes, the contribution of serine/threonine (S/T) phosphatases remains poorly defined. Protein phosphatase 1B (PP1B, a S/T protein phosphatase, has been recently identified by our group as essential for MK development, platelet biogenesis, and hemostatic function. MK-specific PP1B deficiency results in severe macrothrombocytopenia and platelet dysfunction. However, the molecular mechanisms underlying these phenotypes remain unclear.

Aims

To elucidate the molecular mechanisms by which PP1B regulates MK development and platelet production.

Methods

MK-specific PP1B conditional knockout (cKO) mice were generated by crossing Ppp1cb-floxed mice with Pf4-Cre transgenic mice. Bone marrow cells were isolated from the femur, tibia, and ilium of PP1B-deficient and wild-type mice, followed by lineage depletion. Hematopoietic stem and progenitor cells were cultured in thrombopoietin and hirudin-supplemented medium to generate MKs in vitro. MK number, morphology, and ploidy were assessed by flow cytometry and immunofluorescence microscopy. Intracellular signalling and cell-cycle regulators were analyzed by Western blotting. DNA damage and DNA damage response (DDR) signalling were evaluated in control and PP1B-deficient MKs, at baseline and following ultraviolet (UV) stimulation with DNA strand breaks assessed by comet assay.

Results

Our flow cytometric analyses demonstrated that PP1B cKO mice exhibited an increased population of highly polyploid MKs, accompanied by an increased number of MKs in the bone marrow. Despite these changes, major signalling pathways involved in MK development showed minimal differences between PP1B-deficient and wild-type MKs in vitro. These findings prompted us to investigate DNA damage response and cell-cycle regulation in PP1B-deficient MKs. Notably, PP1B-deficient MKs showed increased activation of the DDR pathway and an enhanced DDR following UV-induced DNA damage. Consistent with these findings, preliminary comet assay data demonstrated increased DNA strand breaks in PP1B-deficient cells compared with controls. Furthermore, phosphorylation of CDK1 at Tyr15, an inhibitory modification of CDK1 activity, was markedly increased in both MKs and smaller PP1B-deficient cells.

Conclusion

Our findings identify PP1B as a regulator of megakaryopoiesis and thrombopoiesis and suggest a novel role in DDR signalling, genomic stability, and cell-cycle control.

Poster 49

Effects of the phytosterols β -sitosterol, campesterol, and stigmasterol on the biosynthesis of sex(pro)hormones in steroidogenic H295R cells

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Phytosterols such as β -sitosterol, campesterol, and stigmasterol are widely consumed through plant-based foods. Moreover, high doses can be achieved by consuming fortified foods intended to reduce blood cholesterol levels. Given their close structural similarity to cholesterol, phytosterols may interact with steroidogenic pathways at both the protein and gene expression level, potentially modulating protein abundance and/or enzymatic activity. To date, the effects of phytosterols on the biosynthesis of endogenous sex(pro)hormones remain unknown.

Therefore, the influence of phytosterols on the steroidogenesis of sex(pro)hormones was investigated by incubation of adrenal H295R cells with solvent control, 0.01 μ M - 5 μ M phytosterols and positive controls for induction (1 and 10 μ M forskolin) and inhibition (1 μ M prochloraz) of steroidogenesis at non-cytotoxic concentrations for 48 h. Cell culture supernatants (CCS) were analyzed by GC- and LC-MS/MS for the quantification of 22 steroids, comprising C18 (estrogens, n=7), C19 (n=12), and C21 (n=3) steroids including conjugates.

Furthermore, the impact of phytosterol-induced modulations in the sex(pro)hormone profile on the estrogenic activity of the H295R CCS was evaluated using a modified estrogen receptor alpha-dependent alkaline phosphatase reporter gene assay.

Incubation with β -sitosterol or stigmasterol significantly reduced the total concentration of C21 steroids compared with the solvent control, whereas the total concentrations of C19 and C18 steroids remained unchanged. However, the proportion of bioactive unconjugated estrone and 17 β -estradiol was significantly increased. In addition, an effect on the sulfonation of C19 steroids was observed. β -Sitosterol also significantly increased the concentration of the most potent androgen, dihydrotestosterone. In contrast, treatment with campesterol significantly increased the total concentration of C18 steroids, particularly estrone, compared with the solvent control. Whereas CCS of H295R cells treated with the positive controls significantly decreased (prochloraz, with unconjugated C19 and C18 steroid concentrations reduced by 4%, estrone, – 57%, 5-androstenediol) or increased (1 μ M forskolin, with unconjugated analyte concentrations increased by 136%, dihydrotestosterone, – 1,373%, 16 α -hydroxyestrone) the estrogenic potential of the CCS in the reporter gene assay, the phytosterol-induced changes in the steroid profile did not significantly affect the estrogenic potential of the respective CCS.

Future studies will therefore investigate the effects of phytosterols on the enzyme activities and expression of CYP11A1, sulfotransferases, and steroid 5 α -reductase.

Poster 50

Trispecific single-chain T-cell engaging antibody constructs for immunotherapy of multiple myeloma and epithelial cancers.

Johannes Löffler, Dr. Thomas Bumm

T cell engaging therapies are one of the most prolific areas of cancer research of the last decade. Among them, T cell engaging antibodies offer of-the-shelf opportunities to enable the immune system to attack specific tumour-associated antigens (TAA) and showed already remarkable success in acute lymphoblastic leukemia and multiple myeloma. In solid tumours, the success remains limited as targets are often expressed by tumours and vital tissues alike. As single-targeted approaches already lead to fatal side effects in clinical studies, attacking signatures of multiple TAAs, that are only found on malignant cells but not on healthy tissue might help to overcome this limitation of applying the technology to a broader range of cancers. This work presents the feasibility and in vitro functional evaluation of a novel trispecific, AND-gated single-chain antibody construct, coined "Keylock" antibody. Keylock antibodies were designed to engage T cells only in the presence of two TAA on one target cell (AND-gated) and spare single-TAA positive bystanders. The work showed that these constructs could successfully be produced in mammalian protein expression systems and that the Keylock format was able to selectively induce target cell lysis and T-cell activation against double-TAA positive cells. We designed constructs that target the established multiple myeloma surface proteins CD38 and SLAMF7, as well as further Keylock designs targeting the important epithelial tumour targets EpCAM and HER2. Finally the experimental results were correlated to AI-driven analysis of three-dimensional folding of the corresponding antibody design.

Poster 51

NFAT Fine-Tune NK Cell Maturation, Homeostasis, and Effector Functions

Xiaoli Lou, Yin Xiao, Ali Hassan, Friederike Berberich-Siebelt

NFAT transcription factors (NFATc1, c2, c3), activated via calcineurin and blocked by CsA or FK506, are expressed in NK cells across tissues, as confirmed using Nfatc1-eGFP reporter mice. NKp46⁺iCre-driven deletion of NFAT genes in NK cells reveals that NFAT deficiency promotes NK cell maturation, particularly in the spleen, but reduces total NK cell numbers in the liver and blood. NFAT-deficient NK cells display enhanced proliferation, elevated expression of activation markers, and increased FASL. Functionally, NFATc1-deficient NK cells show stronger cytotoxicity against tumor targets, along with elevated CD107a and IFN- γ secretion, while NFATc1 KO alone reduces IFN- γ output. Together, these findings demonstrate that NFAT factors fine-tune NK cell development, homeostasis, and effector responses.

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Poster 52

Mechanistic Characterization of AGT Clustering in DNA repair: Biochemical, Structural and Single-Molecule Approaches

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A plethora of DNA repair mechanisms protect organisms from DNA lesions caused through exogenous and endogenous sources. The direct damage reversal mechanism of the O6-alkylguanine-DNA alkyltransferases (AGT), also known as O6-methylguanine-DNA methyltransferase (MGMT) in humans, removes highly mutagenic and cytotoxic alkyl lesions from the DNA, and thus also plays an important role in resistance to alkylating chemotherapeutic agents. The removal of the O6-methyl group from guanine involves an auto inactivating irreversible reaction where the alkyl group is directly transferred to the cysteine present in the active site pocket. Although AGT exists exclusively as a monomer in the absence of DNA, it can form cooperative clusters on DNA at high concentrations. However, the molecular mechanisms governing this cooperative clustering, spatial directionality and organization of cluster growth, as well as its physiological role are still not completely understood. This research project aims to elucidate the mechanisms responsible for AGT clustering on DNA. To structure the experimental pipeline, the initial focus is on assessing wild-type and catalytically inactive AGT protein in protein - protein interactions on undamaged DNA and on DNA containing an alkyl lesion, using biochemical and single molecule fluorescence-based approaches such as C-Trap and GETvNA. Furthermore, the structural organization of AGT clusters on DNA will be investigated using cryo-EM, and the physiological relevance of AGT clustering will be studied in a cellular proximity ligation assay. The next phase will focus on studying DNA-binding, enzymatic activity, and assembly properties of selected AGT mutants targeting residues known to contribute to cluster formation. This will provide further validation of how the absence of specific residues affects AGT cluster formation. Finally, A-tract-containing DNA will be used to investigate the preferential binding and organization of AGT clusters using AFM, building on previous GETvNA studies of protein-cluster diffusion. This highly comprehensive and complementary approach will provide new mechanistic insights into the mechanism and role of alkyl lesion dependent AGT clustering and its relevance to DNA repair.

Poster 53

AI-based classification of microsatellite status from endoscopic images in colorectal cancer

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Colorectal cancer (CRC) is diagnosed endoscopically and, depending on the stage, treated endoscopically or surgically. However, therapeutic decisions rely on molecular characteristics that are usually obtained post-hoc from histologic analysis of biopsy specimens, a process that can take several days. The microsatellite status of a tumor is pivotal as lesions with microsatellite instability (MSI) confer a more favorable prognosis and exhibit superior responsiveness to immunotherapy compared with microsatellite stable (MSS) tumors. Artificial intelligence (AI) has the potential to determine MSI status directly from white light images obtained during colonoscopy, thereby expediting clinical decision making.

Methods: A vision transformer that takes an image of a colorectal cancer and classifies its microsatellite status (MSS vs MSI) was trained using 1,091 endoscopic images from 270 CRC patients treated at University Hospital Würzburg between 2014 and 2024. Performance was assessed by accuracy, area under the receiver operating characteristic curve (AUROC), sensitivity, specificity, and negative predictive value. External validation used endoscopic images from four independent centers. EigenCAM method was applied at prediction time to identify the image regions driving each output.

Results: On the internal test set (n = 58, 8 MSI cases) the model reached an AUROC of 0.92 (95% CI: 0.82 - 1.00), accuracy 87.9% (95% CI: 77.1 – 94), sensitivity 75% (95% CI: 40.9 – 92.9), and specificity 90% (95% CI: 78.6 – 95.7). On the external validation dataset (n = 83), the model achieved an AUROC of 0.85 (95% CI: 0.76 - 0.95), accuracy 86.7% (95% CI: 77.8 – 92.4), sensitivity 81% (95% CI: 60 – 89.9), and specificity 88.7% (95% CI: 78.5 – 94.4).

Conclusions: Model performance was preserved across an external multicenter dataset, indicating the potential for real time microsatellite assessment. Prospective validation of the model's performance within routine clinical practice and systematic evaluation of its integration into decision support systems are essential objectives for subsequent studies.

Poster 54

Novel Roles of the Immune Checkpoint Inhibitor CTLA-4 during Cardiac Regeneration

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Ischemic heart disease remains the leading cause of death worldwide. Unlike adult mammals, neonatal mice exhibit a transient capacity for cardiac regeneration that is lost shortly after birth. During this brief period, the induction of myocardial infarction (MI) drives cardiomyocytes towards dedifferentiation, accompanied by a reduced inflammatory immune response. Hence, heart muscle regeneration is enabled by cell-to-cell communication networks of the neonatal cardiac niche. To elucidate the underlying signalling pathways, we analysed published single-cell RNA sequencing data from cardiomyocytes and non-myocytes following myocardial infarction in the regenerative neonatal and non-regenerative postnatal periods. Our analysis revealed the interaction between dedifferentiating and immature cardiomyocytes expressing *Ctla4* and CD80+/CD86+ antigen-presenting cells, suggesting an unexpected function of the immune-regulatory receptor CTLA-4 in the control of cardiomyocyte maturation.

Through in vitro validation with neonatal cardiomyocytes, we observed a sustained immaturity as well as cell cycle re-entry and an increased glycolytic activity upon CTLA-4 activation. In vivo, AAV9-based overexpression of cardiac *Ctla4* induced increased dedifferentiation of cardiomyocytes upon both MI and in sham-operated hearts. These findings suggest that cardiac CTLA-4 not only holds a potential immunomodulatory role but also a key regulatory function in cardiac regeneration by promoting plasticity of cardiomyocytes.

Poster 55

Valence and avidity determine the agonistic activity of anti-TNFR2 nanobody fusion proteins

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We identified several TNFR2-specific nanobodies (Nbs). When formatted as dimeric Fc fusion proteins, these nanobodies exhibited no agonistic activity. To improve activity, we genetically fused one, two, or three copies of the TNFR2-specific Nb:188 to the constant regions of an IgG1 antibody, an Fab fragment, an Fc domain or the trimerization domain of tenascin-C resulting in constructs with 2–12 Nb:188 domains. Constructs with 2 or 3 Nb:188 domains displayed no or minimal activity, while those with 4 and 5 Nb:188 domains demonstrated moderate activity. However, constructs with 6 or more Nb:188 domains exhibited potent agonism, reaching half-maximal TNFR2 activation at concentrations in the low picomolar range. Similarly, hexameric constructs generated with other TNFR2-specific Nb domains demonstrated robust agonism, too. Benchmarking against various other ligand- and antibody-based TNFR2 agonists revealed that the hexameric 3xNb:188-Fc format displays superior specific activity and efficiently expands regulatory T cells (Tregs).

Poster 56

Generation of agonistic TRAILR1-specific nanobody constructs.

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We generated single-domain antibodies (sdAbs, nanobodies) of heavy chain-only antibodies of camelids by immunization of alpacas using a fusion protein of the TRAILR1 extracellular domain. As bivalent Fc fusion protein the TRAILR1-specific nanobodies revealed affinities in the low to sub-nM range, and included TRAIL competing and non-competing ones. None of the TRAILR1 nanobodies showed intrinsic agonistic activity as bivalent Fc fusion protein, but all constructs exerted strong agonism in the presence of FcγR expressing cells. The latter, confirmed the experience, made with conventional bivalent antibodies and soluble TRAIL trimers, that plasma membrane-anchoring empowers per-se inactive or poorly active TRAILR1 binders to trigger strong TRAILR1 activation. Oligomerization, thus increase of valency, is another established principle to confer or enhance agonistic activity of soluble TRAIL and non-agonistic or even antagonistic TRAILR1 or TRAILR2 antibodies. Accordingly. Therefore, we generated various oligovalent TRAILR1 nanobody constructs and indeed obtained this way highly potent TRAILR1 agonists. Worth mentioning, despite not stimulating TRAILR2, these novel TRAILR1 agonists frequently outcompeted on TRAILR1/TRAILR2 double-positive cells various TRAILR1/TRAILR2 cotargeting benchmarks including Aponermin, a clinically approved TRAIL variant, and Eftozanermin, a hexavalent TRAIL variant under investigation in clinical trials.

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Poster 57

Histone chaperone pathways cooperate with ATR/CHK1 signaling to suppress DNA damage and premature mitotic entry

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Faithful genome duplication requires coordinated DNA replication, chromatin assembly, and checkpoint signaling to preserve genome integrity. How replication-coupled chromatin assembly influences the acute cellular response to checkpoint inhibition remains poorly understood. Using a targeted siRNA screen monitoring CHK1 inhibitor-induced DNA damage and premature mitotic entry, we identified replication-coupled chromatin assembly as a determinant of cellular dependence on ATR-CHK1 signaling. Acute depletion of the CAF-1 subunit CHAF1A modestly delayed S-phase progression but caused only limited DNA damage. In contrast, inhibition of CHK1, ATR, or WEE1 in CHAF1A-deficient cells triggered extensive DNA damage, premature mitotic entry, and loss of viability. Transcriptomic analysis revealed preferential activation of B-MYB/Myb-MuvB (MMB) target genes following CHK1 inhibition in CHAF1A-deficient cells, despite only limited changes in promoter accessibility detected by CUTAC. Consistent with a functional role for this transcriptional response, depletion of B-MYB attenuated checkpoint inhibitor-induced DNA damage and premature mitotic entry. Depletion of ASF1 and NAP1L1 similarly enhanced checkpoint inhibitor sensitivity, indicating that increased dependence on ATR-CHK1 signaling is a broader consequence of impaired histone chaperone function. Together, our findings identify replication-coupled chromatin assembly as a determinant of cellular dependence on ATR-CHK1 signaling and establish a functional link between histone chaperone pathways, mitotic transcriptional programs, and the cellular response to checkpoint inhibition.

Poster 58

Activated CD4+ conventional T cells lead to a disruption of the gut barrier

Hannah Maria Nies, Jessica Kümmel, Constanze Binder, Natalie Burkard, Nicolas Schlegel, Johanna C. Wagner

The pathogenesis of inflammatory bowel diseases (IBD), manifesting predominantly as ulcerative colitis and Crohn's disease, is incompletely understood. It is known that genetic predisposition, environmental factors, imbalanced immune reactions, and loss of intestinal epithelial barrier function contribute to the onset of IBD. Mechanisms of the direct interaction between epithelial cells and the immune system remain to be resolved. Here we established a reductive in vitro model as a basis to assess these mechanisms.

The HT29/B6 intestinal epithelial cell line was used. Cells were seeded onto ThinCert® cell culture inserts and grown to confluence in RPMI 1640 medium for 7 days. The inserts were transferred to an ECIS 8-well Transfilter Array (8wTFA) to assess changes in intestinal epithelial barrier properties in transepithelial electrical resistance (TEER) measurements using an ECIS® Z Theta device. Differentiated monolayers were then exposed to pro-inflammatory cytokines (IL1 β , TNF- α , IFN- γ) or co-cultured with activated CD4+ conventional T cells. Continuous TEER measurements were then applied to assess the dynamics of intestinal epithelial barrier changes.

Under control conditions over 48 hours, TEER remained stable compared to baseline. Application of pro-inflammatory cytokines resulted in a loss of TEER to $71 \pm 3\%$ of baseline, which was sustained until the end of measurements. Conventional CD4+ T cells were activated using Dynabeads™ human T cell Activator CD3/CD28 and then co-cultured with the HT29/B6 monolayers. Preliminary results show a loss of TEER to $71 \pm 6\%$ of baseline with T cells on the apical side and loss of TEER to $73 \pm 5\%$ of baseline with T cells co-cultured on the basolateral side.

In summary, we established an in vitro model that successfully reproduces the effects of pro-inflammatory cytokines and co-cultured immune cells. This model provides a valuable tool for investigating in detail the mechanisms of the interaction between activated CD4+ T cells and intestinal epithelial cells.

Poster 59

Single-nucleus RNA-sequencing identifies transcriptional disease signatures in the motor cortex and thalamus of Huntington's disease mice

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Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder resulting in the expression of a mutant huntingtin protein with an expanded polyglutamine tract. This mutation leads to cellular alterations, including huntingtin aggregation, altered gene expression, and impaired proteostasis. HD particularly affects brain regions involved in motor control. Accumulating evidence suggests that impairments in striatal afferents are among the earliest events in the pathological cascade, which precede degeneration of striatal neurons. The two main sources of glutamatergic striatal afferents are the motor cortex and thalamus. However, the pathological changes and the underlying molecular mechanisms remain insufficiently explored.

To identify the vulnerable cell types and disease-relevant molecular pathways in the upstream regions of the striatum, we performed single-nucleus RNA sequencing (snRNA-seq) of the motor cortex and thalamus from the zQ175DN mouse model of HD and wild-type littermate controls at the presymptomatic (6 months) and symptomatic disease stage (14 months). This approach allowed us to obtain a detailed view of all expected cell types and their transcriptional dysregulation. We observed up- and down-regulated transcriptional programs related to cellular stress and identity loss. In the motor cortex, layer 5 pyramidal tract neurons showed the most pronounced transcriptional alterations, including multiple stress responses and compromised cell type identity by increased spermatogenesis and angiogenesis gene expression. In the thalamus, reduced mTORC1 pathway expression was observed in glutamatergic neurons and astrocytes, pointing towards lower growth signaling and higher autophagy. These previously unrecognized patterns of gene dysregulation point to new pathways with potential roles in HD pathogenesis.

Poster 60

Harnessing novel E3 ligases for cancer-specific degradation of oncogenic targets by PROTACs

Napasorn Nilparuk, Katharina Schneider, Hermann Schindelin, Elmar Wolf

Proteolysis-targeting chimaeras (PROTACs) are an emerging class of therapeutics that hijack the cell's protein degradation machinery to eliminate disease-causing proteins. Unlike conventional inhibitors, which block protein function, PROTACs induce degradation of their target protein, thereby modulating proteins previously considered "undruggable." A prominent example is the indirect targeting of the oncogenic transcription factor MYC via its co-factor WDR5. Despite this promise, all PROTACs developed to date rely on the ubiquitously expressed E3 ligases cereblon (CRBN) and von Hippel–Lindau (VHL) to recruit the degradation machinery. Because these ligases are expressed throughout the body, PROTACs built on them lack tissue specificity, increasing the risk of on-target toxicity. Employing an E3 ligase with restricted, cancer-specific expression could help overcome this limitation. This project investigates TRIM72, a RING-type E3 ligase that has not previously been explored for PROTAC development. TRIM72 is an attractive candidate: its structure is known from a prior crystal structure, and DepMap expression data indicate that it is selectively expressed in multiple myeloma. However, no small-molecule ligand has been identified for TRIM72 yet, and such a ligand is a prerequisite for PROTAC design. Our central aim is therefore to discover such ligands and, in doing so, establish TRIM72 as a novel entry point for targeted protein degradation. To this end, we employ fragment-based screening by Nuclear Magnetic Resonance (NMR) spectroscopy, which is sensitive to weakly binding fragments, in combination with X-ray crystallography, which provides atomic-resolution structural detail. These orthogonal approaches allow fragment hits to be identified and validated as starting points for optimisation, with the resulting structural data informing subsequent computationally assisted drug design. Preliminary NMR screening has identified a set of candidate fragments, which are currently being validated by X-ray crystallography.

By expanding the repertoire of E3 ligases available for PROTAC development, this project contributes to more selective and versatile strategies for targeted protein degradation. Establishing TRIM72 as a PROTAC-compatible ligase would provide a new route towards safer, more tissue-restricted therapeutics for MYC-driven cancers and beyond.

Poster 61

Unsupervised Phenotyping of hiPSC-Derived Lung Progenitors Using the cyCONDOR Flow Cytometry Analysis Pipeline

Raymond Okokoh

Human induced pluripotent stem cells (hiPSCs) provide a renewable source for generating airway epithelial cells and offer an attractive platform for studying human airway and lung development and disease. However, differentiation cultures are inherently heterogeneous, and conventional manual flow cytometry gating may overlook intermediate cell states or introduce analyst-dependent bias. Here, we apply cyCONDOR, an unsupervised flow cytometry analysis workflow based on R, to characterise hiPSC-derived lung progenitor populations during airway epithelial differentiation. Lung progenitor identity is assessed using NKX2-1, a key marker of lung epithelial specification, together with EpCAM, indicating epithelial identity, and NGFR, associated with the emergence of airway basal progenitor characteristics.

Flow cytometry data are subjected to quality control, live-cell and singlet selection, dimensionality reduction and unsupervised clustering. The resulting cellular landscape is visualised using approaches including PCA, UMAP and t-SNE, while FlowSOM clustering is used to identify phenotypically distinct populations based on combined NKX2-1, EpCAM and NGFR expression. Marker-expression profiles are subsequently used to interpret the identified clusters and quantify their relative abundance across biological replicates.

This approach is designed to distinguish lung progenitor states that may be difficult to resolve using conventional sequential gating, including epithelial NKX2-1-positive progenitors, NGFR-expressing airway progenitors and potential transitional populations. It also saves time as it can analyse hundreds of fcs files in matter of hours. By converting multiparametric flow cytometry data into reproducible, data-driven cellular phenotypes, cyCONDOR provides a complementary strategy for assessing differentiation efficiency and culture heterogeneity.

Overall, this work demonstrates the potential of unsupervised flow cytometry analysis to improve the characterisation and quality control of hiPSC-derived lung progenitor differentiation and provides a framework that can subsequently be extended to later stages of airway epithelial development.

Poster 62

Abiraterone resistance is associated with epigenetic and metabolic remodeling and increased ferroptosis vulnerability in prostate cancer

Sammy Okutoyi, Oliver Hahn

Resistance to androgen receptor (AR) pathway inhibitors, including abiraterone and enzalutamide, remains a challenge in the treatment of advanced prostate cancer. Cancer cells can adapt to therapeutic pressure through AR alterations and epigenetic reprogramming, contributing to the development of castration-resistant prostate cancer (CRPC). However, these adaptations may also create new therapeutic vulnerabilities. In our study, we investigated whether acquired abiraterone resistance is associated with an epigenetic and metabolic state that could increase susceptibility to ferroptosis.

An abiraterone-resistant VCaP cell model was generated through long-term drug exposure and resistance was verified using viability assays. Western blotting revealed a global reduction of H3K27me3, a repressive histone modification, in resistant cells. CUT&RUN sequencing confirmed an extensive genome-wide loss of H3K27me3 in resistant cells. H3K27me3 levels were restored by either overexpressing EZH2 or knocking down KDM6A in resistant cells, implicating the EZH2-KDM6A axis in this altered chromatin state. Lipidomic analysis showed that resistant cells exhibited a remodeled fatty acid composition, including increased abundance of polyunsaturated fatty acids (PUFAs) associated with susceptibility to lipid peroxidation. Complementary SILAC-based proteomics identified differentially abundant proteins and indicated remodeling of lipid and steroid metabolism. Through Seahorse measurements and mass spectrometry, resistant cells also displayed reduced mitochondrial respiratory capacity and altered cellular iron abundance and speciation. Functionally, abiraterone-resistant cells were more sensitive to RSL3- and erastin-induced ferroptosis and exhibited greater lipid peroxidation, effects that were attenuated by ferrostatin-1.

Together, our findings identify broad H3K27me3 loss, metabolic rewiring and enhanced ferroptosis sensitivity as potentially interconnected features of acquired abiraterone resistance. This ferroptosis vulnerability may present a therapeutic opportunity in therapy resistant prostate cancer. Ongoing studies are investigating whether epigenetic remodeling directly regulates ferroptosis sensitivity and are extending cell-line findings to patient-derived prostate cancer organoid models already established in our laboratory.

Poster 63

From Immunosuppression to Immune Activation: Spatial Profiling–Driven Stratification for Vaccine Therapy in Relapsed HGG

Catharina Ostermann, Mira Heitland, Anna Piro, Mac Dang, Serena Veschi, Matthias Eyrich and Ignazio Caruana

Background: Recurrent pediatric high-grade glioma (pHGG) remains a lethal malignancy characterized by rapid proliferation, molecular heterogeneity, and a profoundly immunosuppressive tumor microenvironment (TME). A critical unmet need is the identification of biomarkers capable of guiding rational immunotherapy selection. High-dimensional spatial profiling using MACSima enables multiplexed, single-cell–resolved mapping of tumor and immune architecture while preserving tissue context, thus providing functional insight beyond conventional histopathology and bulk molecular testing.

Methods: In the HIT-HGG Rez Immunovac trial (Eudra-CT 2013-000419-26 / NCT03879512), patients with relapsed pHGG received tumor lysate–loaded dendritic cell vaccination (DCV) ± immune checkpoint inhibitors (ICI). In this translational analysis, retrospective MACSima profiling was performed on pre-immunotherapy tumor samples collected prior to DCV ± ICI administration, ensuring that spatial signatures reflected the baseline immune ecosystem. Multiparametric analysis characterized immune composition, activation states, macrophage polarization, spatial cell–cell relationships, MHC class I/II expression, proliferative index (Ki67), GFAP expression, and tumor-associated antigens. Integrated molecular profiling included histone mutations and p53 status to identify spatially anchored biomarkers potentially predictive of response.

Results: Baseline spatial mapping revealed an immune-cold TME dominated by myeloid cells (~50%), predominantly M2-polarized macrophages. T cells (~10%) were scarce, spatially dispersed, and frequently isolated from one another. When present, they displayed an exhausted phenotype and physically and functionally constrained from effective tumor engagement. The tumor compartment exhibited aggressive features, including high Ki67, strong GFAP expression, frequent p53 alterations, and H3.3K27M or H3.3G34V/F mutations. Notably, GFAP⁺ tumor cells showed robust MHC class II expression, revealing a potential vulnerability to CD4⁺ T-cell–mediated responses upon immune priming. Following DCV ± ICI, patients demonstrated systemic immune activation, regulatory T-cell reduction, expansion of memory CD4⁺ subsets, and encouraging survival signals.

Conclusions: MACSima-based spatial profiling delineates baseline architectural and functional barriers to immunity in relapsed pHGG. Ongoing data analyses aim to correlate the identified molecular alterations with clinical patient data to determine their clinical relevance. Prospectively implemented, this strategy could enable biologically informed patient stratification and rational upfront therapeutic selection, supporting combinatorial immunotherapy approaches aimed at reversing T-cell exhaustion and converting spatially restricted immunity into durable anti-tumor responses.

Poster 64

Development of an Automated DEL Analysis and Enrichment Tool for High-Throughput DNA-Encoded Library Screening

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Hit identification is a critical step in early-stage drug discovery, involving the screening of large chemical or biological libraries to identify molecules that bind to therapeutic targets and serve as starting points for lead optimization. Traditionally, this process has relied on high-throughput screening (HTS) of physical small-molecule libraries. Although HTS has been highly successful, it is resource-intensive and costly, requiring extensive compound collections, large reagent volumes, and sophisticated robotic infrastructure while exploring only a limited portion of chemical space. DNA-Encoded Library (DEL) technology has emerged as a transformative alternative by covalently linking unique DNA barcodes to individual small molecules, enabling millions to billions of compounds to be pooled and screened simultaneously in a single affinity selection experiment. Following target selection, bound compounds are identified by next-generation sequencing (NGS), allowing efficient hit discovery while substantially reducing experimental cost and material requirements. Consequently, DEL has become an important platform for small-molecule hit identification and lead discovery across diverse therapeutic targets, accelerating early-stage drug discovery while enabling exploration of an unprecedented breadth of chemical space (Brenner & Lerner, 1992; Goodnow et al., 2017; Satz et al., 2022).

Despite its experimental advantages, DEL screening generates massive sequencing datasets whose analysis remains computationally challenging. Accurate hit identification requires barcode decoding, sequencing error correction, compound reconstruction, normalization, enrichment calculation, and visualization. These computational steps are frequently performed using multiple independent scripts or partially manual workflows, reducing reproducibility, scalability, and the efficient interpretation of DEL screening results.

Here, we present an automated DEL analysis and enrichment tool that streamlines the complete computational workflow from sequencing data to enriched hit identification. The pipeline reconstructs three-cycle DEL compounds by assigning BB1, BB2, and BB3 building blocks using annotation tables following barcode decoding and sequencing error correction. Reads containing excessive barcode errors are excluded from downstream analysis, while successfully decoded molecules are retained for quantitative analysis. Compound abundances are normalized using counts per million (CPM), followed by enrichment analysis through comparison of target selections against native libraries and experimental controls, including denatured protein and empty-bead selections, thereby improving discrimination between specific binders and nonspecific background. The workflow has been successfully applied to large-scale three-cycle DEL screening datasets comprising hundreds of millions of sequencing reads. To facilitate interpretation of DEL datasets, the workflow automatically generates enrichment tables, corrected scores, and publication-ready visualizations, including building-block enrichment distributions, violin plots, and interactive three-dimensional scatter plots for structure–activity relationship (SAR) exploration. Designed for execution on high-performance computing (HPC) environments, the workflow enables scalable and reproducible analysis while substantially reducing manual data processing.

This integrated computational framework provides a robust, reproducible, and scalable platform for DEL data analysis, supporting efficient hit prioritization, rapid interpretation of screening results, and accelerated small-molecule drug discovery.

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Poster 65

NELLY: A transparent deep learning framework for patient-centric drug response prediction and prioritization

Christian Peralta Viteri, Nadja Harnischfeger, Lili Szabo, Stefan Hartmann, Kai Kretzschmar

Introduction

Over the past decade, several machine learning methods have been developed to predict patient responses to anti-cancer drugs. Despite methodological advances, they present limited translational relevance for personalized medicine. The main limitations of current state-of-the-art methods can be summarized by their inflated performance, limited transparency and scarce validation in patient-derived material.

Methods

Cancer drug response prediction models use gene expression and chemical information to learn patterns associated with treatment sensitivity. For training, we used gene expression data from Cell Model Passport and pharmacogenomic data from Genomics of Drug Sensitivity in Cancer. To improve transparency, we developed NELLY, a deep learning model incorporating a dynamic weighting mechanism (DWM). This module enables extraction of patient-specific gene-level attributions associated with predicted patient response.

Models were trained and evaluated using a cell-line-blind 10-fold cross-validation to assess performance in unseen cell lines. Additionally, we applied recommendation-based methods, including Precision@K and NDCG@K to assess models' ability to rank the most effective drug for a given patient. For independent validation with patient material, we have assembled a pan-cancer patient-derived organoid pharmacogenomic atlas. The use of this fully independent dataset allowed us to evaluate generalization performance to clinically relevant samples.

Conclusion

We demonstrated that NELLY outperforms state-of-the-art models in generalization to cancer organoid data, achieving the best performance for patient-specific drug prioritization. Additionally, DWM-derived gene attribution analysis uncovered resistance-associated programs related to cancer cell plasticity, thereby adding mechanistic interpretability beyond drug ranking.

Poster 66

In-vitro test platform for CAR-Macrophage validation

Luca Pauline Pölzer

Although immune checkpoint therapy (ICT) has transformed cancer treatment, approximately half of melanoma patients still experience toxicity or fail to respond to therapy. Resistance to ICT in solid tumors has been associated with an increased prevalence of immunosuppressive (M2) tumor-associated macrophages (TAMs), which can be induced by molecules such as glucocorticoids (GCs). Recent studies have shown that macrophages engineered with chimeric antigen receptors (CAR-M) can actively target and combat solid tumors. However, CAR-M have demonstrated limited persistence and an unstable pro-inflammatory, anti-tumor (M1) phenotype *in vivo*.

Our previous study has shown that TAM-specific deletion of the glucocorticoid receptor (GR) promotes an M1-like macrophage phenotype, enhances tumor cell phagocytosis and improves therapeutic responses in an ICT-resistant murine melanoma model. GR loss in TAMs was also associated with enhanced CD8+ T-cell activity, indicating that GR signaling contributes to therapy resistance through coordinated effects on both myeloid and lymphoid compartments. Based on these findings, we hypothesize that GR-deficient CAR-Ms will exhibit enhanced antitumorigenic properties and improve responses to ICT *in vivo*.

To test this hypothesis, we combine CRISPR-mediated GR knockout (GR KO) with the expression of a melanoma-specific CAR in human macrophages, thereby generating GR-KO CAR-Ms. We first characterize their polarization state by flow cytometry and assess the stability of the M1-like phenotype following exposure to different M1- and M2-polarizing stimuli. We then evaluate their antitumor activity using an *in vitro* test platform comprising human melanoma cell lines (A375, G361, and SK-MEL-28) and murine melanoma cell lines (YUMM1.7 and B16F10) engineered to constitutively express mCherry and luciferase. In co-culture with GR-deficient or GR-proficient CAR-Ms, phagocytic and cytotoxic activity is quantified by flow cytometry and immunofluorescence microscopy. Finally, the engineered CAR-Ms are combined with OT-I T cells to determine their capacity to promote antigen-specific T-cell activation.

Together, this platform will enable the functional characterization and preclinical validation of GR-deficient CAR-Ms as a novel therapeutic strategy, thereby linking mechanistic insights into GR-mediated immunosuppression with the development of more effective treatment approaches for patients with PD-1-resistant melanoma.

Poster 67

Synthesis and investigation of chiral polycyclic heteroaromatic hydrocarbons incorporating main group elements

Mykyta Radchenko, Daniel Volland, Jan Niedens, Prof. Agnieszka Nowak-Król

Helicenes are ortho-fused polycyclic (hetero)aromatic compounds with a screw-shaped and chiral geometry. Their unique optical and electronic properties have led to applications in asymmetric catalysis, optoelectronic devices, sensors, nanocages, semiconductors, chiral emissive materials and circularly polarized organic light-emitting diodes (CP-OLEDs). Moreover, helicenes are gaining attention for their potential use in spintronic devices due to their ability to exhibit chiral-induced spin selectivity. BN-doping of organic scaffolds, achieved by replacing C–C bonds with B–N bonds, leads to significant changes in their electronic structure and molecular geometry, resulting in altered and often improved optical properties. Consequently, BN-doped PAHs have attracted attention for use in organic field-effect transistors (OFETs) [1], organic light-emitting diodes (OLEDs) [1], and organic photovoltaics (OPVs) [2]. The incorporation of azaborole rings into helicenes has also been shown to enhance their luminescence quantum yields [3–4], as demonstrated for a unique azaborathiahelicene synthesized in our group [5]. Importantly, the presence of the thiophene rings in this structure induced chalcogen-chalcogen interactions in the solid state, which can be beneficial for organic electronics [6–7]. Motivated by these results, we designed an azaborathiahelicene, in which one thiophene ring is replaced with pyrrole. This poster presents the synthesis and optoelectronic properties of this unique compound.

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Poster 68

G6b-B controls the transition from progenitors to mature megakaryocytes through transcriptional and cell cycle regulation.

Hadiqa Raees, Maximilian Englert, Duy Thai Duong Le, Gabriel H.M. Araujo, Bernhard Nieswandt and Zoltan Nagy.

Megakaryocyte (MK) maturation requires coordinated exit from proliferative progenitor states and activation of lineage-specific transcriptional programs, yet the signaling mechanisms governing this transition remain incompletely understood. G6b-B is an ITIM-containing immunoreceptor essential for platelet production. Loss of G6b-B in mice and humans leads to severe thrombocytopenia and myelofibrosis. While its requirement for MK maturation is established, the downstream mechanisms remain unclear. Here, we show that loss of G6b-B in mice disrupts progression along the MK lineage. Single-cell RNA sequencing revealed a shift toward immature cell states, with expansion of MK progenitors (MKPs) and reduction of mature MKs. Transcriptional changes were strongly polarized across differentiation: MKPs displayed broad upregulation of gene expression, whereas mature MKs exhibited widespread downregulation. Differentially expressed genes in HSPCs and MKPs were enriched for transcription factors and gene regulatory network analysis (SCENIC) identified enhanced activity of stem cell-associated regulons (e.g. Mecom, Cux1). These changes were accompanied by enrichment of cell cycle and DNA damage response programs based on gene ontology analysis.

These findings were supported by altered progenitor composition in vivo, including expansion of LSK cells, reduction of LK populations, and increased leukocyte counts in peripheral blood, consistent with altered lineage output. Phosphoprotein profiling analysis (Sciomics) further linked these changes to dysregulated cell cycle control, including altered phosphorylation of the cell cycle regulator B-MYB and reduced levels of the cell cycle phosphatase CDC25B in platelets. Together, these findings identify G6b-B as a regulator of the transition from proliferative progenitor states to mature megakaryocytes by coordinating transcriptional and cell cycle programs.

Poster 69

Soluble chemokine gradients direct splenic type 1 dendritic cell migration along perivascular tracks

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Migration of dendritic cells (DCs) is essential for their ability to relay antigenic information from peripheral tissues and instruct antigen-specific T cells within lymphoid organs. DC migration is driven by CCR7-dependent haptotaxis along immobilized CCL21 gradients toward lymphatic vessels, enabling transport to draining lymph nodes. How DC migration is organized within the spleen, an organ specialized in blood surveillance, has remained unclear. Here, using high-resolution confocal microscopy and functional perturbations, we show that splenic cDC1 migrate from the red pulp to the white pulp by following perivascular tracks surrounding arterioles – the same pathways used by lymphocytes entering from the bloodstream. Strikingly, this migration is guided not by immobilized chemokine cues but by long-range gradients of soluble CCL19 and cleaved CCL21. Although these chemokines are produced within the white pulp, they are distributed along perivascular spaces toward the venous sinuses of the red pulp establishing a directional chemotactic gradient. Thus, splenic DC migration operates through a paradigm fundamentally distinct from lymphatic DC trafficking, relying on chemotaxis rather than haptotaxis. Together, our findings define the anatomical pathways and molecular mechanisms underlying DC migration within the spleen and reveal how vascular organization shapes immune cell positioning.

Poster 70

CD28 costimulation, but not C40L ligation, enhances T cell recall responses to SARS-CoV-2 antigen in whole blood

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Apart from innate immunity adaptive immunity, i.e. T and B cells, substantially contribute to the control of viral infections. Apart from killing virally infected cells, activated CD4⁺ and CD8⁺ T cells mediate anti-viral immunity by IFN γ . For optimal IFN γ secretion by both recently activated effector as well as memory T cells, CD28 costimulation is required, which is readily provided by antigen-presenting cells in secondary lymphoid organs.

In humans, however, peripheral blood is the compartment of choice when it comes to assessing T cell immunity. Here, the most direct and best compatible approach is to work with whole blood to determine the degree of cellular immunity against SARS-CoV-2. For the Elecsys® IFN γ release assay (IGRA) SARS-CoV-2 this is the case as anti-viral T cells are first stimulated in whole blood with a mixture of more than 200 peptides derived from SARS-CoV-2 proteins followed by detection of IFN γ in the plasma. We blocked CD28 costimulation during the IGRA with a Fab fragment of an anti-CD28 mAb and compared the results to those obtained with cultures stimulated in the presence of a blocking anti-CD40L mAb or in the absence of mAb. Our data indicate that optimal IFN γ release into the plasma required CD28 costimulation, while CD40L ligation was dispensable. Similarly, optimal IL-2 secretion also depended on CD28 costimulation, which we measured in a multiplex assay together with IL-4, -5, -6, -10, -17A, -22, TNF and, again, IFN γ .

Our data, thus, show that despite the big differences in cellular composition between whole blood and secondary lymphoid organs IFN γ and IL-2 secretion by T cells in whole blood also depended on CD28 costimulation. Therefore, T cell activation in whole appears to follow very similar rules to that in secondary lymphoid organs.

Poster 71

Targeting platelet GPVI with the function-blocking JAQ1Fab attenuates myocardial ischemia-reperfusion injury

Matthias Richter, Stefano Navarro, Kristina Lorenz, Bernhard Nieswandt

Background:

Myocardial infarction (MI) triggers a thrombo-inflammatory response involving platelet activation, inflammation, and microvascular dysfunction, which can further aggravate myocardial injury following reperfusion. Given the contribution of platelets to these processes, targeting platelet activation represents a promising approach to limit thrombo-inflammatory myocardial injury. Previous studies showed that the collagen and fibrin(ogen) receptor Glycoprotein VI (GPVI), expressed only on platelets and their progenitors megakaryocytes, plays a key role in the development of thrombo-inflammatory conditions. The anti-murine GPVI monoclonal antibody JAQ1, which downregulates the receptor in vivo, established GPVI as a safe anti-thrombotic and anti-inflammatory target. In the context of myocardial ischemia-reperfusion (I/R), JAQ1IgG treatment improved myocardial microperfusion and reduced infarct size, highlighting GPVI as a potential target for limiting thrombo-inflammatory myocardial injury. However, JAQ1IgG causes transient thrombocytopenia in mice and long-term depletion from the platelet surface, thereby limiting its therapeutic application. Aims: To achieve functional GPVI inhibition without receptor depletion, the Fab fragment JAQ1Fab which, like JAQ1IgG, blocks GPVI-dependent platelet activation and collagen-induced aggregation, should be evaluated in a model of myocardial I/R injury.

Results:

Here, we investigated whether functional GPVI inhibition with JAQ1Fab protects against myocardial I/R injury. Treatment with JAQ1Fab resulted in reduced scar formation two weeks after I/R, indicating attenuated myocardial injury. These findings support the concept that selective blockade of platelet GPVI can modulate thrombo-inflammatory processes following myocardial reperfusion. To further validate the anti-GPVI treatment in a large animal model an anti-porcine GPVI antibody was developed.

Conclusions:

By blocking mouse GPVI and reducing scar formation in a myocardial I/R injury model using JAQ1Fab the therapeutic potential of targeting GPVI in thrombo-inflammatory settings were further validated. To better evaluate the translational potential of GPVI inhibition, Fab fragments targeting human GPVI will be investigated in a humanized GPVI mouse model. This approach will allow us to assess whether pharmacological inhibition of human GPVI can similarly reduce myocardial injury and infarct size in a thrombo-inflammatory setting.

Poster 72

Deciphering Host-Pathogen Interactions in BKPyV Infection Using a Human Bladder Organoid Model

Janina Rieger, Maria Guedes, Clara Husser, Marcel Schwinger, Charis Kalogirou, Nicole Fischer, Carmen Aguilar

BK Polyomavirus (BKPyV) represents a significant threat to immunocompromised individuals, particularly recipients of bone marrow and kidney transplants. Following primary infection, BKPyV establishes lifelong latency in the urogenital tract, with seroprevalence exceeding 80% in the adult population¹. Loss of immune surveillance can lead to viral reactivation and severe clinical disease. In bone marrow transplant recipients, BKPyV infection of the bladder causes polyomavirus associated hemorrhagic cystitis, a frequent and debilitating complication for which effective therapeutic options are limited. In contrast to BKPyV kidney infection, the molecular mechanisms controlling BKPyV infection in the bladder remain poorly defined. To address this knowledge gap, we use human-derived bladder organoids and bladder cancer cell lines to investigate virus-host interactions and to identify host determinants of viral entry, replication, and persistence. Immunofluorescence analysis of early and late viral protein expression revealed infection-associated alterations in nuclear architecture. Comparative analysis of BKPyV infection efficiency showed heterogeneous susceptibility among bladder cancer cell lines, whereas the differentiated bladder organoid based model exhibited a high infection efficiency. Notably, the bladder organoid-based model supported productive BKPyV infection and released infectious viral progeny. Single-cell RNA sequencing of BKPyV-infected organoids revealed preferential infection of a highly proliferative subset of bladder epithelial cells. Even among cells with low infection levels, a strong antiviral response was evident. Ongoing studies aim to further define host factors and molecular decision points that shape BKPyV infection dynamics in the bladder epithelium. Together, these results validate the human bladder organoid-based model as a physiologically relevant model of BKPyV bladder infection and support their use for the identification of host pathways likely to be relevant to therapeutic intervention.

Poster 73

Spatial Transcriptome Profiling in follicular lymphoma and distinct subgroups

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Follicular lymphoma (FL) is highly heterogeneous both in biological and clinical features. These features are known to depend on the tumor microenvironment (TME), which varies among different defined FL subgroups. Therefore, a deeper understanding of the interaction with the TME in FL in general, but also with focus on distinct FL subgroups is warranted.

For this purpose, whole transcriptome gene expression profiles of selected regions of interest including all cell types as well as regions of interest limited to B-cells, T-cells, and follicular dendritic cells (FDCs) were analyzed in the germinal center (GC) and in the extra-tumoral mantle/marginal/interfollicular zones (M/M/IFZ) of 66 formalin-fixed and paraffin embedded FL samples with information on t(14;18) using Nanostring GeoMx Digital Spatial Profiling. Data analysis was accomplished using the commonly used GeoMxTools R-pipeline and the more recently published R package standR (Spatial transcriptomics analyses and decoding in R), which revealed more meaningful data by reducing batch effects, compared to the GeoMxTools R-pipeline in a recent comparison study with non-tumor GeoMx datasets.

In line with previous findings, this study identified significant batch-associated variability in our FL GeoMx data using the common GeomxTools R-pipeline. Applying the standR workflow substantially reduced technical variation. The improved sensitivity of StandR also made it possible to identify biologically more meaningful differences in gene expression profiles between t(14;18)-positive and t(14;18)-negative FL, with previously identified differentially expressed molecular markers (e.g., NF- κ B-associated genes and CD10) serving as a reference and as confirmation of the validity of this approach. Preliminary analyses revealed substantial differences among different compartments, cell types and FL with and without t(14;18), which are currently analyzed in more detail. In conclusion, this spatial whole transcriptome approach provides insights into FL heterogeneity and pathogenesis that cannot be obtained using bulk whole transcriptome approaches. Moreover, the results from this approach will serve as a guide for further, more detailed high- and low-plex analysis at the single-cell and protein level to further improve the understanding of the oncogenic signaling between the tumor and its microenvironment in different molecular and clinical FL subgroups.

Poster 74

Development of a New Immunotherapy Treatment Based on Gene-Modified Adaptive NK Cells for the Refractory Lymphomas

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CD30 is an established therapeutic target in Hodgkin lymphoma and other CD30⁺ malignancies, supporting the development of CD30 cellular therapies. While Chimeric Antigen Receptor (CAR) T-cell approaches have shown encouraging activity, their clinical implementation remains challenged by patient-specific manufacturing, product variability, prolonged production timelines and the risk of functional exhaustion. Natural killer (NK) cells represent an attractive off-the-shelf alternative with a favourable safety profile; however, NK-cell products are often limited by suboptimal persistence and in vivo expansion. Adaptive NK cells (aNK), which arise in response to cytomegalovirus exposure, display a mature CD57⁺KIR⁺NKG2A⁻ phenotype, high expression of the activating CD94/NKG2C receptor, enhanced IFN- γ production and potent antibody-dependent cellular cytotoxicity. These features provide a strong biological rationale for exploiting aNK cells as a fitter platform for CAR immunotherapy.

Here, we developed a GMP compatible strategy to generate CAR aNK cells targeting CD30⁺ malignancies. aNK expansion was achieved using a monoclonal antibody directed against CD94, capable of blocking the dimerization of CD94 with the inhibitory receptor NKG2A while promoting engagement with the activating molecule NKG2C. In parallel, we generated a 4th generation CD30.CAR incorporating CD28 and OX40 costimulatory domains to enhance activation and persistence. To further support aNK fitness, the construct was implemented to secrete a CD94 single fragment variable region (scFv) and IL-15, thereby combining receptor level functional reinforcement with cytokine survival support.

Using feeder-free culture conditions, we established a scalable manufacturing workflow compatible with clinical translation. Initial validation in bulk NK cells demonstrated robust CAR expression and potent cytotoxic activity against multiple CD30⁺ lymphoma cell lines, resulting in near-complete tumor elimination in long-term in vitro co-culture assays. Compared with conventional CD30.CAR NK cells, 4th-generation CD30.CAR NK cells showed markedly increased IFN- γ secretion ($p < 0.001$). Production of both IL15 and the CD94-scFv was confirmed at protein level. We then optimized the engineering of aNK cells, achieving $82 \pm 11\%$ while preserving their adaptive phenotype.

Overall, this study established a clinically oriented platform that combines the intrinsic fitness of aNK cells with the specificity and potency 4th-generation CD30.CAR. By integrating feeder-free GMP compatible expansion, high-efficiency genetic engineering, IL15 support, CD94/NKG2C pathway reinforcement and CD94/NKG2A blocking, this approach provides a promising off-the-shelf cellular therapy for CD30⁺ malignancies and addresses key limitations of current autologous CAR-T-cell strategies.

Poster 75

Evangelia Sarantaki

Poster 76

Design, Synthesis and Pharmacological Evaluation of a Light-Responsive μ -Opioid-Receptor Ligand as an Efficacy Switch

Thomas Schidelko, Marthe Vandeputte, Christophe Stove, Michael Decker

A novel photoswitchable ligand of the μ -opioid-receptor (MOR) derived from the highly potent agonist etonitazene was prepared by an azo-extension approach. Etonitazene was chosen as the starting molecule, since it has a chemical structure which enables azo-extension and shows >1,000 the potency of morphine in animals, therefore tolerating reduction in affinity due to azo-extension. The resulting photo-nitazene (UW-MD-591) exhibits optimal photophysical properties, including efficient and reversible photoisomerization (PSS365 nm (trans/cis) = 06:94 and PSS530 nm (trans/cis) = 82:18), no photo-fatigue and a long thermal half-life ($t_{1/2}$ > 16 hours). Photopharmacological evaluation of UW-MD-591 in a MOR β -arrestin 2 recruitment assay suggested that the compound may act as a “trans-on” efficacy switch with potency staying in the same range for both photoisomers. The photo-nitazene UW-MD-591 could serve as a photopharmacological tool compound for potential light-mediated reversible spatiotemporal control of MOR signaling.

Poster 77

Functional Characterization of Plakoglobin in Colorectal Cancer

Leonie Schnappinger

Metastatic colorectal carcinoma remains a leading cause of cancer-related mortality worldwide despite substantial advances in surgical and systemic therapy as well as early detection measures. Cell adhesion proteins are increasingly recognized as regulators of tumor progression and metastatic dissemination. However, the contribution of plakoglobin, a protein found in desmosomes and adherens junctions, remains incompletely understood. Recent data suggests it plays a broader role in tumorigenesis, particularly by modulating proliferative and migratory behavior of intestinal epithelial cells.

We aimed to investigate the functional role of plakoglobin in colorectal cancer with particular emphasis on WNT signaling, cell adhesion and migration.

Human und murine colorectal cancer cell lines as well as murine organoids with plakoglobin knockdown and overexpression were analyzed using molecular and functional assays including western blotting, rt-qPCR, flow cytometry and migration, proliferation and adhesion assays. For these studies, a panel of constitutive and inducible shRNA constructs targeting plakoglobin (JUP) in human and murine colorectal cancer cells and organoids was established. Preliminary characterization demonstrated efficient knockdown without detectable effects on WNT signaling. Human cells with plakoglobin knockdown showed increased cell migration and decreased cell adhesion in different functional assays.

Our findings suggest a potential role for plakoglobin in regulating adhesive and migratory properties in colorectal carcinoma cells. Additional analyses will be conducted to further characterize these observations.

Poster 78

Exploring the Conformational Landscape of the E. coli Virulence Factor Lymphostatin

Ronja Schneider

Escherichia coli pathotypes such as enteropathogenic (EPEC) and enterohemorrhagic E. coli (EHEC) rely on a diverse arsenal of virulence factors to manipulate host immune responses. Among these, Lymphocyte Inhibitory Factor A (LifA), also known as lymphostatin, inhibits mitogen-activated lymphocyte proliferation and proinflammatory cytokine production, thereby facilitating gut colonization. The first three-dimensional map of lymphostatin at 23-Å resolution revealed an elongated, L-shaped molecule. Recent single-particle cryo-electron microscopy (cryo-EM) studies extended these findings, showing that LifA adopts a multi-domain architecture comprising six distinct domains connected by five flexible linker regions. Furthermore, LifA exhibits notable structural plasticity across different buffer conditions: under acidic to near-neutral conditions (pH 4.0 and 6.5), two distinct structural states (conformation 1 and 2) were initially observed, whereas slightly basic conditions (pH 8.0) revealed a third state (conformation 3).

However, whether these structural shifts are directly driven by pH, influenced by buffer compositions during purification, or caused by other factors remains unclear. Because LifA is highly prone to proteolytic degradation, modifying purification workflows presents a significant challenge. To avoid structural changes induced by multiple pH adjustments from a pH 6.5 baseline, and to analyze an additional pH value for direct comparison, a novel, end-to-end purification strategy conducted entirely at pH 7.5 was established to preserve full-length LifA. Preliminary cryo-EM analysis and 2D class averages of samples purified directly at pH 7.5 demonstrate that conformation 3 is the predominant structural state in this case. Minor populations corresponding to conformation 1 and/or 2 were also observed, though further data collection is required for high-resolution 3D reconstruction and precise differentiation. No additional conformational states were identified in the initial 2D classes.

Poster 79

Maintenance of genome stability by SFPQ in YAP-induced replication stress

Viviane Schnell, corrected by Prof. Dr. Stefan Gaubatz

High levels of proliferation driven by oncogenes often lead to replication stress. One of these oncogenes is YAP (Yes-associated protein) and its homologue TAZ, the downstream effectors of the Hippo signaling pathway. YAP and TAZ are activated in many human tumors and have been identified as potent inducers of tumor cell proliferation. YAP globally increases transcriptional activity, thereby causing conflicts between the transcription and replication machineries (TRCs). When combined with CHK1 inhibition, YAP activation induces extensive DNA damage. CHK1, the downstream effector kinase of the ATR-CHK1 pathway, limits replication stress by restricting origin firing and delaying mitotic entry.

To identify factors that protect YAP-expressing cells from replication stress, a small-scale siRNA screen was performed, identifying the multifunctional RNA binding protein SFPQ as a protective factor. SFPQ depletion increased DNA damage during S-phase in YAP-expressing cells and induced a pronounced G1 arrest.

To determine how SFPQ protects YAP-expressing cells from replication stress and DNA damage, non-transformed MCF10A cells with inducible SFPQ depletion and YAP expression were generated. When R-loop formation was assessed by DNA:RNA immunoprecipitation (DRIP)-qPCR and DNA:RNA hybrid dot blot analysis, SFPQ depletion caused a modest increase in global R-loop levels, which was further enhanced by YAP activation. However, no significant changes in R-loop accumulation were detected at selected genomic loci previously reported to be affected by SFPQ depletion.

To investigate the cause of the G1 arrest, 53BP1 nuclear bodies were analyzed as markers of under-replicated DNA. SFPQ-depleted cells displayed an increase in 53BP1 nuclear bodies, which was further exacerbated by YAP activation, suggesting increased replication-associated genome instability. Finally, to assess the therapeutic potential of targeting SFPQ, cells were treated with increasing concentrations of a CHK1 inhibitor. Colony formation assays demonstrated that SFPQ-depleted, YAP-expressing cells were more sensitive to CHK1 inhibition than control cells. Similar effects were observed in multiple cancer cell lines, where combined SFPQ depletion and CHK1 inhibition resulted in increased replication stress and DNA damage compared with either treatment alone. These results indicate that SFPQ plays an important role in maintaining genomic stability and targeting SFPQ might be a promising therapeutic strategy for YAP-driven cancers.

Poster 80

Optogenetic Control of cGMP by Enzyme Rhodopsins: From Microbial Photoreceptors to Genetic Tools

Jiachen Shi, Markus Bender, Christine E. Gee, Georg Nagel, Shiqiang Gao

cGMP is a key second messenger that controls diverse cellular functions. Enzyme rhodopsins provide an attractive solution to manipulate cGMP dynamics because they combine a retinal-based light sensor with an effector domain that directly synthesizes or degrades cyclic nucleotides. Among them are two cGMP-related enzyme rhodopsins from evolutionarily distant microbial eukaryotes that have become, or have the potential to become, valuable optogenetic tools.

The rhodopsin guanylyl cyclase BeCyclop derives from the aquatic fungus *Blastocladiella emersonii*, where the native rhodopsin–guanylyl cyclase is part of the light-sensing machinery of motile zoospores and contributes to phototactic behavior. In contrast, RhoPDE was identified in the choanoflagellate *Salpingoeca rosetta* as a rhodopsin-phosphodiesterase fusion protein with light-regulated cyclic nucleotide hydrolysis, showing a clear preference for cGMP over cAMP. BeCyclop and RhoPDE provide complementary tools for bidirectional optical control of intracellular cGMP levels. We have applied BeCyclop in combination with functional assays to analyze cGMP generation and degradation with high temporal precision. Our recent work further indicates that retinal uptake efficacy can affect the activity of these rhodopsins, providing an additional handle to tune their photochemical and enzymatic performance. This chromophore dependence is especially relevant for the future optimization of RhoPDE as a research tool, as it shows relatively high dark activity. We are currently testing the possibility of using luciferase as an internal light source to activate BeCyclop, providing a chemical activation strategy for in vivo applications where light cannot be delivered easily.

Poster 81

Tumor-draining lymph node immunosuppression supports distant metastatic seeding by limiting systemic immunity

Andrea Stabile, Greta Mattavelli, Moutaz Helal, Dalia Sheta, Andreas Beilhack, Angela Riedel

Triple-negative breast cancer (TNBC) is characterised by a high risk of distant metastasis, yet the mechanisms by which the tumour-draining lymph node (TDLN) influences systemic immune changes remain incompletely understood.

This study builds on evidence that TNBC remodels the TDLN through a TLR4–CCL2/CCL7–CCR2 axis, driving the recruitment of immunosuppressive monocytes and impairing local T-cell function, therefore creating the so-called pre-metastatic niche that will support the onset of distant metastasis. The stromal and immune compartment of the TDLN can be remodelled by local targeted therapies, such as immune checkpoint blockade or TLR4 inhibitor treatment, in order to revert the lymph node immunosuppression leading a reduced metastatic burden.

The objective of this study is to determine whether alterations in the TDLN, induced by the tumour, affect the trafficking of immune cells to distant organs and contribute to systemic immune dysregulation that would therefore lead to an increased distant metastatic proliferation.

Utilising a photoconvertible Dendra2 mouse model, we can track the cells residing in the TDLN and observe their subsequent migration to peripheral organs during the progression of tumours, focusing on the immune profiling of these.

The mice are orthotopically injected in the mammary fat pad with TNBC cells, 24 hours before the endpoint of the experiment the inguinal LNs are photoconverted through the skin using a 405nm laser probe in order to photoconvert from the green form to the red form the Dendra2 protein expressed by all CD45+ cells. Spectral flow cytometry and scRNA sequencing are used to characterise the recirculating populations and define their phenotypic changes following tumour development. Additionally, to detect the presence of micro metastasis at the pre-metastatic time point and eventually visualise the recirculating immune population associated with these, we tested whether the model would be suitable for light sheet focal microscopy (LSFM).

Identifying the cellular and molecular mechanisms driving systemic immune dysregulation would uncover potential therapeutic targets within the lymph node microenvironment and evaluate targeted interventions that could reduce the impact of distant metastases.

Poster 82

Isoform-Specific Targeting of VEGFR1 Reduces Hepatic Steatosis

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Metabolic dysfunction-associated steatotic liver disease (MASLD) has become a major public health concern due to its rapid rise in prevalence. However, to date, disease pathogenesis and targeted treatment options remain incompletely understood. In recent years, research has begun to investigate vascular endothelial growth factor receptor 1 (VEGFR1) as a potential contributor to and therapeutic target for MASLD. VEGFR1 functions as a negative regulator of vascularization in vascular homeostasis. It exists as a soluble (sVEGFR1) and a membrane-bound isoform (mVEGFR1). VEGFR1 binds VEGFA and limits its bioavailability to VEGFR2. Recently, modulation of fat uptake has also been reported as a downstream effect of VEGFR1 signaling. Because its decoy function in angiogenesis and VEGFR1 signaling are interconnected, the effects of VEGFR1 activation on metabolic disorders can be difficult to distinguish. We used a novel mouse model in which VEGFR1 expression is fully redirected to the sVEGFR1 isoform to investigate the effects of VEGFR1 signaling loss while maintaining its sVEGFR1 decoy function. We analyzed visceral white adipose tissue (vWAT) and the liver after 12 weeks of HFD feeding. Our results show that loss of mVEGFR1 and the shift toward sVEGFR1 ameliorated hepatic steatosis and increased fat deposition in vWAT. qPCR analysis of the liver and isolated hepatocytes identified decreased expression of fatty acid transporters and Vldlr, along with indicators of increased β -oxidation, as potential key factors in reducing hepatic lipid accumulation. In vitro experiments identified placental growth factor as a driver of VEGFR1-mediated hepatic lipid uptake. In an exploratory study, we furthermore found decreased serum VEGFR1 levels in morbidly obese patients with MASLD. In summary, this study identifies VEGFR1 as a promising drug target for reducing hepatic steatosis and as a biomarker for MASLD.

Poster 83

The Role of BCOR-ITD in kidney and neural development and the formation of pediatric tumors

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BCOR is a part of the Polycomb Repressive Complex 1.1. (PRC1.1), which belongs to a larger family of PRCs. These complexes play a crucial role in maintaining naïve pluripotency and restricting cell lineages during embryonic development. They carry out this function by modifying histones modification through methylation and ubiquitylation, which leads to the transcriptional silencing of developmental genes.

An internal tandem duplication (ITD) in the BCOR exon 15, which encodes a protein interaction domain, has been identified as the sole driver of various pediatric tumors, including clear cell sarcomas of the kidney and high-grade neuroepithelial tumors. To improve our understanding of the molecular mechanism underlying BCOR-ITD-driven tumor formation, an embryonic stem cell (ESC)-based in vitro model system was developed that enables a Cre-recombinase-mediated switch from wild-type BCOR to BCOR carrying the ITD mutation (BCOR-ITD). This system enables the precise timed activation of the ITD mutation during neural and renal differentiation.

To gain an initial understanding of the impact of the ITD mutation on undifferentiated ESCs, we first analyzed changes in global histone methylation levels upon ITD activation by CUT&RUN. Our results thus far show that H3K4 trimethylation (me3) levels at specific gene loci are altered in response to ITD activation. RT-PCR expression analyses revealed that differentially methylated genes were also expressed at higher levels. GATA6, one of these genes, has previously been shown to be upregulated upon BCOR knockdown, indicating that the ITD mutation may represent a loss-of-function mutation. Further global analyses comparing the methylation and expression patterns of BCOR knockout ESCs with those of BCOR-ITD ESCs will clarify this fundamental question.

In in vitro differentiation assays, BCOR-ITD ES cells initially exhibited normal neural differentiation potential. However, upon prolonged culture, colonies of cells were observed that started to overgrow the differentiated cell cultures. Morphologically, the cells within these colonies exhibited features of undifferentiated neuroepithelial cells. We hypothesize that these cells represent immortalised or transformed neural progenitor cell types. Further transcriptomic and functional assays are currently in progress to confirm this.

Poster 84

Strain-Dependent Pro-Inflammatory Responses of Human Fibroblast-like Synoviocytes to European *Borrelia burgdorferi sensu lato* Isolates from Lyme Arthritis Patients

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Introduction: Lyme borreliosis, caused by spirochetes of the *Borrelia burgdorferi sensu lato* (Bbsl) complex, is the most prevalent tick-borne disease in North America and Europe. A frequent late manifestation is chronic inflammatory Lyme arthritis (LA). In a subset of patients, synovial inflammation persists or worsens despite adequate antibiotic therapy, resulting in antibiotic-refractory LA (ARLA). While LA in North America is caused mainly by *Borrelia burgdorferi sensu stricto* (s.s.), multiple Bbsl genospecies contribute to the disease burden in Europe, suggesting additional complexity in host-pathogen interactions. Although highly inflammatory strains of *B. burgdorferi* s.s. have been associated with ARLA in North America, the inflammatory potential of European *Borrelia* genospecies and strains remains poorly characterised.

Objectives: This study aimed to investigate the pro-inflammatory potential of European Bbsl strains isolated from LA patients and to characterise the immune responses of human fibroblast-like synoviocytes (HFLS) following infection.

Material & Methods: Seven European *Borrelia* strains representing the heterogeneity of Bbsl genospecies circulating in Germany were directly isolated from knee joint punctures of patients with LA. HFLS from healthy controls were infected in vitro with the diAerent isolates. Host transcriptional responses were analysed by bulk RNA sequencing, followed by principal component analysis and gene set enrichment analysis. In parallel, cytokine and chemokine concentrations in cell culture supernatants were quantified by multiplex bead-based immunoassay.

Results: Distinct *Borrelia* isolates induced pronounced pro-inflammatory transcriptional responses in HFLS. These responses were characterised by strong upregulation of inflammatory mediators including IL6, CXCL1, CXCL3, CXCL6, CXCL8, CXCL10 and CCL2, accompanied by enrichment of interferon-gamma response pathways and downregulation of MYC target gene sets. Importantly, the transcriptional activation of pro-inflammatory cytokines and chemokines was reflected by increased protein secretion into the cell culture supernatant, as demonstrated by measurements of IL-6, CXCL1, CXCL8, CXCL10 and CCL2. No genospecies-specific diAerences in the immune response of HFLS were observed. Instead, substantial variability in inflammatory potential was detected at the strain level.

Summary: European *Borrelia* isolates exhibit marked strain-dependent diAerences in their capacity to induce inflammatory responses in HFLS, indicating that pathogenicity is primarily driven by strain-specific rather than genospecies-specific characteristics. The induction of an IFN-driven, highly inflammatory phenotype in HFLS may represent an initial step to the pathogenesis of ARLA. Further studies are required to elucidate the underlying mechanisms of host-pathogen interaction, including bacterial adhesion and the potential secretion of additional pathogen-associated molecular patterns.

Poster 85

Real-Time AI-Based Ergonomics Monitoring to Reduce Musculoskeletal Strain in Endoscopists: A Pilot Validation Study

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Introduction: Work-related musculoskeletal disorders are reported in as many as 62.5% of endoscopists, driven largely by sustained bad postures and poorly configured equipment. No validated tool currently exists to objectively track posture and deliver corrective feedback during an endoscopy procedure.

Aims: Validation of PostureMeister, an AI-driven posture monitoring system, in a simulated endoscopy room for its ability to detect ergonomic equipment misconfiguration and quantify resulting musculoskeletal risk postures.

Methods: A depth camera tracked body landmarks and equipment position for five participants performing four standardized postures under five different configurations for monitor and bed (optimal, monitor too low/high, bed too low/high). AI-detected landmarks, monitor/bed height classification, and neck/trunk angles were validated against manual reference annotation. **Results:** Body landmarks were detected reliably overall (92.3%), with excellent agreement against the manually annotated landmarks. Monitor height was classified with 100% accuracy ($\kappa = 1.00$), and bed height with 79% accuracy ($\kappa = 0.459$), with no directional misclassifications. Neck angle stayed near neutral under optimal conditions ($+0.7^\circ \pm 6.0^\circ$) and shifted significantly with a low monitor ($+8.2^\circ \pm 3.9^\circ$, $p < 0.001$) or high monitor ($-5.9^\circ \pm 3.4^\circ$, $p < 0.001$), while bed misconfiguration produced smaller but still significant shifts. Trunk angle changes remained small across all conditions. **Conclusion:** PostureMeister reliably detects ergonomically unfavorable monitor and table positioning and precisely quantifies resulting neck strain, supporting its potential to flag musculoskeletal risk postures in real time during endoscopy. Future work will validate the system during real procedures and incorporate a validated ergonomic risk score such as Rapid Entire Body Analysis.

Poster 86

Characterizing the potency and selectivity of novel phosphoglycolate phosphatase inhibitors

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Targeting cellular glucose metabolism is a therapeutic strategy in numerous diseases, including autoimmunity, chronic inflammation, (cardio)metabolic diseases and cancer. Metabolite repair enzymes may represent an alternative target class for selective metabolic inhibition, but suitable pharmacological tools to test this concept are lacking. Phosphoglycolate phosphatase (PGP) is a glycolysis-associated metabolite repair enzyme of the haloacid dehalogenase (HAD) superfamily. PGP dephosphorylates and thereby repairs the erroneous glycolytic metabolites 2-phospho-L-lactate and 4-phosphoerythronate. An accumulation of these metabolites inhibits glycolysis or pentose phosphate pathway flux, respectively. PGP activity is therefore essential to prevent a gridlock in central carbon metabolism under conditions of increased metabolic flux.

We aim to develop potent and selective small-molecule PGP inhibitors to target pathological glucose metabolism. Our initial discovery of the first PGP inhibitor, CP1, has shown that this enzyme is druggable. We have subsequently conducted extensive structure-activity relationship studies based on alternative chemical scaffolds.

Here, we present aspects of the biochemical characterization of novel PGP inhibitor candidates. The potency of these compounds was assessed by determining their half-maximal inhibitory concentration (IC₅₀) values in phosphatase activity assays, using recombinantly expressed, highly purified human PGP. The selectivity of these compounds was then assessed in biochemical activity assays, using a panel of other purified phosphatases.

Our approach has identified compounds that inhibit PGP with high selectivity and low-nanomolar affinity (< 20 nM). These findings represent a significant advance in PGP inhibitor development. The potential of these compounds for further preclinical studies is currently under evaluation.

Poster 87

Mapping and Targeting Functional Binding Sites on p97 by Fragment-Based Drug Design

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The AAA+ ATPase p97 (VCP) is a central regulator of cellular protein homeostasis, functioning as a segregase that remodels ubiquitinated substrates through ATP-dependent conformational changes. It plays key roles in ER-associated degradation (ERAD), autophagy, and protein quality control. Due to its involvement in maintaining proteostasis, p97 is essential for the survival of stressed and rapidly proliferating cells, making it an attractive target, where its inhibition induces proteotoxic stress and apoptosis [1]. Additionally, disease-associated mutations like L198W in p97 are linked to disorders such as multisystem proteinopathy [2].

p97 activity is tightly controlled by a network of cofactors that bind primarily to the N-domain and inter-domain interfaces, regulating substrate specificity and functional outcomes. These regulatory regions provide opportunities for selective therapeutic intervention beyond direct ATPase inhibition. In this work, we employed a fragment-based drug discovery approach combined with X-ray crystallography to identify and characterize novel binding sites on p97, including cofactor interaction sites, interface regions, and the ATPase domain. Fragment hits were validated using ligand-based NMR, bio-layer interferometry (BLI), and grating-coupled interferometry (GCI). Fragment optimization was guided by molecular docking and molecular dynamics simulations, employing fragment growing, linking, and merging to improve binding affinity and selectivity.

By integrating structural, biophysical, and computational approaches, this work reveals new insights into p97 regulation and enables the development of selective, mechanism-based modulators.

References

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Poster 88

Wavelength and intensity of light affect the orientation behavior of *Drosophila*

Romita Trehan, Kimia Soltani and Hannah Haberkern

Visual cues are essential for animal navigation. *Drosophila melanogaster* is a capable navigator, dispersing over kilometres using straight-line orientation guided by external visual cues. Depending on the cues available, flies employ different strategies: for example, *menotaxis*, where they maintain a stable heading relative to a cue such as the sun or an intensity gradient, and *phototaxis*, moving directly toward or away from a light source. *Menotaxis* requires functional EPG cells, which act as an internal compass by tracking the fly's heading. *Phototaxis*, on the other hand, persists even without functional EPG cells. The compass is informed by upstream visual input carrying information like the intensity, contrast, and wavelength, from the photoreceptors through the anterior visual pathway to ring neurons, which converge onto the EPG cells. Navigation behaviour depends on how effective a cue is, and cue effectiveness is itself influenced by these same parameters. How they shape orientation behaviour, however, remains less understood.

Here we examine how orientation responses change with wavelength and intensity in wild-type and EPG-silenced flies, using a closed-loop 2D virtual-reality setup in which a tethered fly walks on a ball. We presented a panorama with a bright and a dark side and systematically varied edge definition, from a sharp boundary, through progressively blurred edges, to a smooth intensity gradient with no clear edge. This stimulus protocol was presented in white, green, blue, and cyan light, along with blue light at a higher intensity.

We found that strategy choice depended on both the edge definition of the stimulus and its spectral composition. While the edge definition influenced the choice between *phototaxis* and *menotaxis*, wavelength and intensity influenced whether flies oriented toward or away from the bright side, and white light produced responses not predicted by its individual components. Silencing EPG neurons removed this flexibility, leaving flies *phototactic* regardless of intensity.

Together, these results show that the spectral properties of a visual cue determine not only how well they orient themselves, but also the direction of that response, and that switching between strategies depends on an intact compass. A natural extension in the future would be to ask whether specific ring neuron populations encode particular wavelengths, using targeted perturbation and functional imaging.

Poster 89

The integrity of platelet TLR2 is comparably preserved under cold and room temperature storage of platelet concentrates

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Beyond hemostasis, platelets are also involved in immunological processes, which have to be considered for strategies to optimize storage conditions of platelets concentrates (PC). For example, platelets bear the toll-like receptor 2 (TLR2), crucial for the recognition of bacterial antigens. However, it is unclear how platelet TLR2 integrity is affected by different storage temperatures of PC. Therefore, this study investigated TLR2 expression, distribution and function in platelets from PC either stored at room temperature (RT, 22-24 °C) or cold temperature (CT, 4-6 °C) for 5 days.

Apheresis-derived PC were initially stored at RT. On day 1 of storage, samples were drawn from PC for platelet analysis. Afterwards, PC were split into two parts, one stored at RT and the other at CT until day 5, when samples were drawn again for analysis. Flow cytometry was used to determine TLR2 surface expression, TLR-stimulated uptake of fibrinogen or TLR2 ligand Pam3CSK4, ROS production and CD40L expression. Visualization of TLR2 on the platelet surface and its distribution in intracellular compartments was performed using transmission electron microscopy and high-resolution fluorescence microscopy. TLR2-stimulated secretion of platelet chemokines was measured by immunoassays.

Surface expression and intracellular distribution of TLR2 were unchanged after 5 days, independent from temperature. TLR2 stimulation remarkably induced CD40L expression and the uptake of fibrinogen or the internalization of TLR2 ligand Pam3CSK4, regardless of storage temperature. TLR2-induced ROS production was comparably decreased by 50 % in Pam3CSK4-stimulated platelets, when stored for 5 days at RT and CT. The capacity of TLR2-mediated chemokine secretion including RANTES, NAP-2, PF4, PDGF or sCD40L was similarly preserved on day 5 at both temperatures. TLR2 integrity is comparably preserved under storage of PC at RT or CT for 5 days, as indicated by TLR2 expression, distribution and related functional effector mechanisms. In addition to hemostatic function, it is imperative to include the analysis of platelet immune functions in studies addressing the optimization of PC storage conditions.

Poster 90

Neuronal toxicity and recovery from early bortezomib-induced neuropathy: blood-nerve barrier dysfunction without dorsal root ganglion damage

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Background: Use of the proteasome inhibitor bortezomib (BTZ) is highly effective in the treatment of multiple myeloma. However, its long-term use is limited by the fact that most treated patients develop painful polyneuropathy. In some of the treated patients, pain resolves with time; in others, it persists, with the underlying mechanisms poorly understood. One prerequisite for neural toxicity is the ability of a drug to penetrate the blood-nerve barrier.

Methods: We analysed the pathways involved in early BTZ-induced polyneuropathy development and its resolution in rats and in patients with myeloma using pain behaviour, barrier analysis, transcriptomics, immunohistochemistry, and clinical data.

Results: One cycle of BTZ elicited transient tactile and cold allodynia in rats. Transcriptomic signature and network analysis revealed regulation of circadian, extracellular matrix, and immune genes within the nerve and modest changes in the dorsal root ganglia. Recovery processes resealed the small molecule leakiness of the perineurial barrier, reversed axonal swelling, and normalised fibre density in the skin. Expression of the microtubule-associated cytoskeletal protein cortactin matched this process in the perineurium. Netrin-1 as a known barrier sealer was also upregulated in pain resolution in nerve and skin.

Conclusions: In summary, our data demonstrate that early bortezomib toxicity targets mainly peripheral nerves and indicate that pain resolution could be supported by protective growth factors such as netrin-1 for remodelling of the extracellular matrix and neuronal barriers.

Poster 91

Molecular Characterization of Wnt Signaling Regulation in the Metacestode of *Echinococcus multilocularis*

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Background: Alveolar echinococcosis (AE), caused by the proliferative metacestode tissue of *Echinococcus*

multilocularis, is among the most severe zoonotic diseases. Therapeutic options are limited: curative surgical resection is rarely feasible, while the chemotherapeutic agent albendazole is only parasitostatic, as parasitic stem cells are insensitive to treatment and capable of regenerating damaged tissue. Against this background, parasitic stem cells and the signaling pathways regulating them represent promising targets for novel therapeutic approaches. The Wnt signaling pathway is of particular interest, as it plays a central role in regulating the posteriorized metacestode tissue, which lacks an anterior pole and body axes during the infectious stage. Knockdown experiments of β -catenin demonstrated that this key Wnt signaling factor is essential for metacestode formation. The Wnt pathway is also well characterized in cancer research, where it plays a central role in the proliferation of colorectal carcinomas. In human cells, the cofactor CBY1 (Chibby) has been identified as an antagonist of the β -catenin/TCF interaction, thereby modulating Wnt signaling. Previous work by the Brehm group has already demonstrated the direct interaction between β -catenin and TCF/LEF in *E. multilocularis*.

Objectives: The aim of this study is to analyze the canonical Wnt signaling pathway downstream of β -catenin,

with a particular focus on its interaction with TCF/LEF and the functional role of the Chibby ortholog (EmuJ_000117800).

Materials & Methods: The chibby gene was analyzed bioinformatically using previously available genomic and transcriptomic data, including an assessment of trans-splicing. Expression patterns of EmChibby and TCF/LEF were investigated by in situ hybridization in metacestode tissue. Protein-protein interactions between EmChibby and β -catenin were examined using yeast two-hybrid assays. Functional analyses are currently being performed using RNA interference.

Results: Nested PCR confirmed that EmChibby undergoes trans-splicing. In situ hybridization revealed expression of EmChibby and TCF/LEF in metacestode cell populations, including stem cells. Yeast two-hybrid analyses demonstrated a direct interaction between EmChibby and β -catenin, which was restricted to the predicted binding domain of β -catenin. Larger fragments resulted in unspecific interactions in control experiments.

Conclusion: These results provide initial insights into the regulation of Wnt signaling in the metacestode of *E. multilocularis* and identify EmChibby as a potential modulator of β -catenin activity. Ongoing RNAi experiments will further clarify its functional role.

Poster 92

Soluble Adhesion Molecules and Functional Outcome in Moderate-to-Severe Acute Ischemic Stroke

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Background: Secondary brain injury after acute ischemic stroke (AIS) involves neuroinflammatory processes characterized by endothelial activation, leukocyte recruitment, and disruption of the blood-brain barrier (BBB). Adhesion molecules expressed by endothelial and immune cells contribute to leukocyte adhesion and transmigration across the vascular endothelium, thereby contributing to the post-ischemic inflammatory response. Experimental targeting of specific adhesion molecules can reduce leukocyte infiltration and subsequent tissue damage, supporting their relevance to post-ischemic injury. Proteolytic shedding of membrane-bound adhesion molecules generates soluble forms detectable in the circulation, which may reflect activation of these pathways. However, the prognostic relevance of soluble adhesion molecules and their potential to provide insight into clinically relevant adhesion pathways after AIS remain poorly understood.

Methods: Patients with moderate-to-severe AIS (admission NIHSS score ≥ 6 points and/or indication for mechanical recanalization) were included in this prospective single-center study. Serum concentrations of ten soluble adhesion molecules (CD44, ICAM-1, ICAM-2, NCAM, ALCAM, VCAM-1, E-selectin, P-selectin, L-selectin, PECAM-1) were measured in samples obtained within 48 h after symptom onset and analyzed according to sampling time (≤ 24 h vs. 24-48 h). Functional outcome was assessed at 3 months (± 14 days) by telephone interview using the modified Rankin Scale (mRS), with poor outcome defined as mRS ≥ 3 . Discrimination for poor outcome was assessed by receiver operating characteristic (ROC) analysis. Associations with poor outcome were evaluated using multivariable logistic regression with log₁₀-transformed adhesion molecule concentrations, adjusted for age, admission ASPECTS, NIHSS at 24 h after admission, and recanalization therapy (yes/no).

Results: Between July 2020 and September 2022, 270 patients were included [n=141 (52%) female, median age 79 years (IQR: 66-84), median NIHSS score on admission 13 (IQR: 8-17), median ASPECTS on admission 8 (IQR: 6-9)], 217 (80%) underwent mechanical thrombectomy and 117 (43%) intravenous thrombolysis. In samples obtained within 24 h of symptom onset, CD44 (AUC 0.70, 95% CI: 0.60-0.81, $p=0.0007$) and ALCAM (AUC 0.72, 95% CI: 0.61-0.82, $p=0.0003$) showed moderate discrimination for poor 3-month outcome, whereas discrimination by the other adhesion molecules was limited. After multivariable adjustment, only CD44 (OR 2451, 95% CI: 22-818847), and ALCAM (OR 49, 95% CI: 3-1169) remained associated with poor 3-month outcome. However, ROC analyses for protein concentrations in blood sampled 24-48 h after stroke onset showed no significant discrimination for CD44 (AUC 0.57, 95% CI: 0.48-0.67, $p=0.1561$), ALCAM (AUC 0.56 95% CI: 0.46-0.66, $p=0.2427$), or any other adhesion molecule. Consistent with these findings, associations of CD44 (OR 10.9, 95% CI: 0.4-377.4) and ALCAM (OR 7.1, 95% CI: 0.5-117.0) with 3-month outcome were no longer evident.

Conclusion: Circulating CD44 and ALCAM were associated with poor 3-month outcome in patients sampled within 24 h after AIS, but not in those sampled 24-48 h after symptom onset. Despite limited prognostic performance, these findings support further investigation of CD44- and ALCAM-related adhesion processes in the initial phase after AIS.

Poster 93

Characterization of Cell-Surface GD2 Expression as a Prerequisite for GD2-directed CAR T-Cell Co-Cultures in Glioblastoma Patient-Derived Organoids

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Objective: Therapeutic options for glioblastoma (GBM) remain limited, in part due to pronounced inter- and intratumoral heterogeneity. Targeting an antigen that is broadly and consistently expressed across tumor cells may help to overcome this challenge. The oncofetal disialoganglioside GD2 represents a promising target for CAR T-cell therapy. Its high and largely homogeneous cell-surface expression has been reported in 8/8 patient-derived H3K27M-mutant diffuse midline glioma culture models (Mount et al., 2018). However, the extent and heterogeneity of GD2 expression in GBM remain to be determined. Patient-derived organoids (PDOs) provide a preclinical model that preserves relevant features of tumor heterogeneity and can additionally be used to investigate interactions between GBM cells and GD2 CAR T-cells in vitro. Therefore, this study aimed to characterize GD2 expression and its inter- and intratumoral heterogeneity across GBM PDOs and to establish initial conditions for PDO-GD2 CAR T-cell co-culture, including E:T ratio and morphological readouts.

Methods: Seventeen PDOs were generated from eight patients to assess inter- and intratumoral heterogeneity. Three tumor regions were sampled from three patients, two regions from three patients, and one region from two patients. Following dissociation into single-cell suspensions, cells were stained with an APC-conjugated anti-GD2 antibody, with corresponding isotype controls, and analyzed by flow cytometry using a NovoCyte Penton. For co-culture experiments, PDOs were incubated with GD2 CAR T-cells at an effector-to-target (E:T) ratio of 1:1. Untreated PDOs and PDOs co-cultured with non-transduced T-cells served as controls. Morphological changes were monitored using the Axion Biosystems Omni system.

Results : GD2 expression was detected on the majority of cells across all PDOs, with the proportion of GD2-positive cells ranging from 74.9 % to 99.8 % (mean: 92.8 %, SD $\pm$ 8.3 %). Preliminary analysis of PDOs derived from different tumor regions of one patient showed consistently high GD2 expression, with a mean proportion of GD2-positive cells of 96.7 % (SD $\pm$ 3.5 %). Following co-culture with GD2 CAR T-cells, PDOs progressively lost their compact structure within 24-72 h and exhibited a disintegrated morphology.

Conclusion: The consistently high proportion of GD2-positive cells across GBM PDOs supports GD2 as a promising target antigen for CAR T-cell therapy despite inter- and intratumoral heterogeneity. Moreover, the observed morphological changes following co-culture with GD2 CAR T-cells demonstrate the feasibility of the PDO-based co-culture model. Further studies should incorporate functional readouts to distinguish tumor cells from CAR T-cells, assess tumor cell viability and CAR T-cell proliferation, and evaluate different E:T ratios to determine conditions that retain anti-tumor activity.

Poster 94

Key signaling pathways underlying CD56-mediated NK-cell activation by *A. fumigatus*

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Background: *Aspergillus fumigatus* is a ubiquitous environmental fungus that is efficiently eliminated by healthy individuals but can cause invasive pulmonary aspergillosis (IPA) in immunocompromised patients. Natural killer (NK) cells, identified by the expression of CD56, play an important role in antifungal defense by releasing lytic granules that damage *A. fumigatus* hyphae and producing chemokines that recruit additional immune cells. CD56 binds to galactosaminogalactan (GAG), a polysaccharide component of the fungal cell wall, thereby promoting NK-cell activation. Blocking CD56 reduces this response, indicating that CD56 functions as a fungal recognition receptor. However, the intracellular signaling pathways activated downstream of CD56 in NK cells remain poorly understood. This study investigated the signaling mechanisms involved in GAG-mediated NK-cell activation using pharmacological inhibitors and characterized CD56-dependent signaling pathways with potential relevance beyond antifungal immunity.

Methods: NK cells were isolated from peripheral blood mononuclear cells obtained from healthy donors and stimulated overnight with interleukin-2 (IL-2). The cells were then pretreated with inhibitors targeting Src-family kinases (PP2), Pyk2 (PF-431396), phosphoinositide 3-kinase (PI3K; Wortmannin), or actin polymerization (cytochalasin D). Following pretreatment, NK cells were co-cultured with *A. fumigatus* hyphae or GAG as well as the respective inhibitors. NK-cell activation was evaluated by flow cytometry and ELISA-based analysis of chemokine production.

Results and Conclusion: Inhibition of Src-family kinases, Pyk2, or PI3K reduced CD56 downregulation and impaired NK-cell activation, as demonstrated by decreased CD69 expression, degranulation, and chemokine release. In contrast, cytochalasin D increased CD56 surface expression while enhancing NK-cell activation and chemokine production. These findings suggest that actin dynamics regulate the surface localization of CD56 but are not required for downstream signaling. Overall, CD56-dependent NK-cell activation involves a coordinated signaling network comprising Src-family kinases, PI3K, and Pyk2. By contrast, actin dynamics appear to primarily regulate CD56 surface expression and localization rather than the downstream activation response.

Poster 95

Molecular Investigations of Stem Cell Self-Renewal and Differentiation in the Fox Tapeworm *Echinococcus multilocularis*

Johannes Walter

The fox tapeworm *Echinococcus multilocularis* causes alveolar echinococcosis, a potentially fatal disease characterized by infiltrative growth of metacestode tissue within the organs of the intermediate host. This growth is driven by totipotent somatic stem cells that are essential for parasite regeneration, tissue renewal, and expansion.

Using a single-cell RNA sequencing atlas generated by our group, we identified several stem cell subpopulations as well as populations of differentiating progenitor cells in addition to differentiated cell types. Here, we aim to identify markers of these progenitor states and experimentally characterize their temporal development.

Candidate genes derived from the scRNA-seq dataset are analyzed by whole-mount in situ hybridization and EdU pulse-chase experiments. Time-dependent colocalization of EdU with progenitor markers allows us to track the transition from proliferating stem cells toward differentiating cell states. Selected candidates are further investigated by RNAi and direct inhibitor treatment to assess their functional relevance.

Preliminary results reveal EdU-positive cells that progressively acquire progenitor marker expression over time, consistent with the expected characteristics of differentiating progenitor cells. However, the analyzed markers also show low-level expression in other cell populations. This suggests that the identified progenitor cells may represent a transient differentiation state rather than a clearly defined and stable cell population. Functional analyses using RNAi and inhibitor treatment have so far not resulted in a clearly detectable phenotype.

Ongoing experiments aim to resolve the dynamics of progenitor differentiation using combined EdU pulse-chase approaches with progenitor and differentiation markers. A detailed understanding of stem cell maintenance and differentiation in *E. multilocularis* may help identify new potential therapeutic targets for alveolar echinococcosis.



Poster 96

Jakob Weilbach

Poster 97

The Dual Ligand: BTN2A1 Immunomodulates Human V γ 9V δ 2 T Cell Response

Michelle Wolf [1], Lara Vugrek [1], Carlotta von Klopotek [1], Lisa Starick [1], Thomas Winkler [2], Carrie R. Willcox [3], Benjamin E. Willcox [3], Mohindar M. Karunakaran [1], Thomas Herrmann [1] [1] Institute for Virology and Immunobiology, Julius-Maximilians Universität Würzburg, Germany [2] Division of Genetics, Department Biology, Friedrich-Alexander-Universität, Erlangen-Nürnberg, Germany [3] Department of Immunology and Immunotherapy, University of Birmingham, Edgbaston, Birmingham, United Kingdom

V γ 9V δ 2 T cells are a major subset of $\gamma\delta$ T cells, comprising 1-5 % of human peripheral blood T lymphocytes. These cells detect cancerous cells and cells infected by pathogens. They respond to phosphoantigens (PAg), phosphorylated intermediates of the isoprenoid synthesis of microbial or host origin via butyrophilins (BTN) expressed by target cells. The binding of PAg to the B30.2 domain of BTN3A1 functions as a molecular glue initiating the intracellular interaction between the B30.2 domains of BTN2A1 and BTN3A1. This complex of BTN2A1 homodimers and BTN3A heterodimers alter the BTN topology, resulting in the interaction of the V domains of BTN2A1 and BTN3A2 with the V γ 9V δ 2 T cell receptor (TCR). In this instance, the T cell receptor undergoes a conformational change, thereby facilitating the binding of the BTN tetrameric complex.

In 2020, BTN-knockout (BTNKO) experiments in HEK293T cells revealed BTN2A1 as a mandatory compound for PAg-mediated activation of V γ 9V δ 2 T cells and a direct ligand to the V γ 9-chain of the $\gamma\delta$ TCR. BTN2A1 expression in BTN2A1-knockout (BTN2A1KO) HEK293T cells by retroviral gene transduction reconstituted the PAg response only partially while the BTN2A1 transduced HEK293T cell reduced the original PAg response, suggesting a suppressive effect depending on the BTN2A1 overexpression. This finding was confirmed by human tumor lines of different origin in a PAg-dependent human V γ 9V δ 2 TCR-transduced murine reporter system (53/4 $\gamma\delta$ TCR MOP hybridoma cells). Experiments with BTN2A1 mutants demonstrated that this effect relates to the BTN2A1-IgV domain but not to its TCR binding. Furthermore, the suppression appears to be PAg-specific, as it was not observed in the activation $\alpha\beta$ T cells or α CD3 stimulation assays. However, the immunosuppressive fashion was not determined for BTN2A1 overexpression in human Raji B cell lymphoma.

We suggest that BTN2A1-mediated immunosuppression as a kind of escape mechanism in solid tumors due to distinct glycosylation pattern of BTN2A1. While tumor cell types display an increased expression of diverse BTN molecules, we hypothesize that the equilibrium of BTN2A1 and BTN3A plays a crucial role in the strength of V γ 9V δ 2 T cell response and varies between the cell types. Therefore, the investigation of the affected BTN expression levels and the cell-specific equilibrium is of interest for the development of V γ 9V δ 2 TCR or BTN-targeting tumor therapies.

Poster 98

Visualizing bacterial structure and dynamics with the optimized fluorescent protein mChartreuse

Ruilan Xu, Christoph Spahn

Fluorescent proteins are widely used to label and monitor target proteins in living cells. However, most were developed for eukaryotic systems and often performed poorly in prokaryotes. Fraikin et al. recently developed a palette of fluorescent proteins (FPs) optimized for bacterial expression [1]. In this work, I evaluated mChartreuse by chromosomally labeling bacterial RNA polymerase (RNAP) and compared its brightness and photostability with two commonly used green FPs, mNeonGreen and sfGFP. mChartreuse proved to be the brightest and most photostable of the three, and the mChartreuse-labeled RNAP strain showed similar viability to wild-type *E. coli* MG1655. Furthermore,, mChartreuse accurately labeled various bacterial proteins in both cytoplasmic and periplasmic environments. Additionally, mChartreuse was anchored to the inner membrane by fusing a synthetic peptide, WALP-D, to its C-terminus [2]. Cells expressing mChartreuse–WALP-D exhibited homogenous membrane fluorescence in live cells and stable patterns after fixation, suggesting potential applications in reference structures for fluorescence and super-resolution imaging. Overall, these results establish mChartreuse as a bright and photostable fluorescent protein for reliable bacterial protein labeling and high-quality imaging across multiple cellular contexts.

[1] N. Fraikin, A. Couturier, R. Mercier, and C. Lesterlin, ‘A palette of bright and photostable monomeric fluorescent proteins for bacterial time-lapse imaging’, *Sci. Adv.*, vol. 11, no. 16, Apr. 2025, doi: 10.1126/sciadv.ads6201.

[2] M. Peschke et al., ‘Distinct Requirements for Tail-Anchored Membrane Protein Biogenesis in *Escherichia coli*’, *mBio*, vol. 10, no. 5, pp. e01580-19, Oct. 2019, doi: 10.1128/mBio.01580-19.

Poster 99

Engineering Channelrhodopsins for Low-Light Growth and Efficient Green-Light Activation in Plants

Heng Zhang, Dirk Becker, Shiqiang Gao

Abstract: Optogenetic tools enable precise spatiotemporal control of cellular activity, but their application in plants is often limited by unintended activation under ambient white light. To address this problem, we expressed a low-light-sensitivity anion channelrhodopsin in Arabidopsis. Transgenic plants grew normally under low ambient white light, while the actuator remained efficiently activated by stronger green light, providing a promising tool for studying anion-dependent signaling under less restrictive growth conditions. In parallel, we generated a panel of calcium-conducting channelrhodopsin mutants and evaluated their Ca^{2+} selectivity in *Xenopus* oocytes under blue-light stimulation. Several variants showed improved Ca^{2+} selectivity compared with their parental constructs. Their action spectra and light sensitivities are currently being characterized under plant-relevant illumination conditions. Together, these results advance the development of plant-compatible optogenetic tools with reduced ambient-light activation and improved control of anion and Ca^{2+} signaling.

Poster 100

The role of MFSD2A in the blood-nerve barrier during neuropathic pain

Huan Zhou, Franziska Schütz, Elena Häring, Julia Metz, Heike L. Rittner, Beate Hartmannsberger

The blood–nerve barrier (BNB) protects peripheral nerves, and its disruption contributes to neuropathies [1]. Barrier integrity relies on tight junction proteins such as claudin-5 that maintain tight junctions (paracellular pathway) and very low levels of transcytosis (transcellular pathway). In the blood-brain barrier (BBB) the major facilitator superfamily domain-containing protein 2a (MFSD2A) is a key regulator and suppresses caveolae-mediated transcytosis [2], which is associated with plasmalemma vesicle-associated protein (PLVAP). It is unclear if MFSD2A regulates transcytosis in the BNB, as well. In this study, we investigated BNB integrity and the expression of MFSD2A and transcytosis-associated proteins in two pain models: reserpine-induced pain and chronic constriction injury (CCI).

We hypothesize that MFSD2A regulates caveolae-mediated transcytosis in the BNB and might contribute to pain resolution in neuropathic pain conditions.

Research aim: To investigate MFSD2A expression and transcytosis-associated protein expression in the BNB in pain model animals.

Poster 101

Unravelling the functional roles and interplay of platelet GPV and CLEC-2 in inflammation

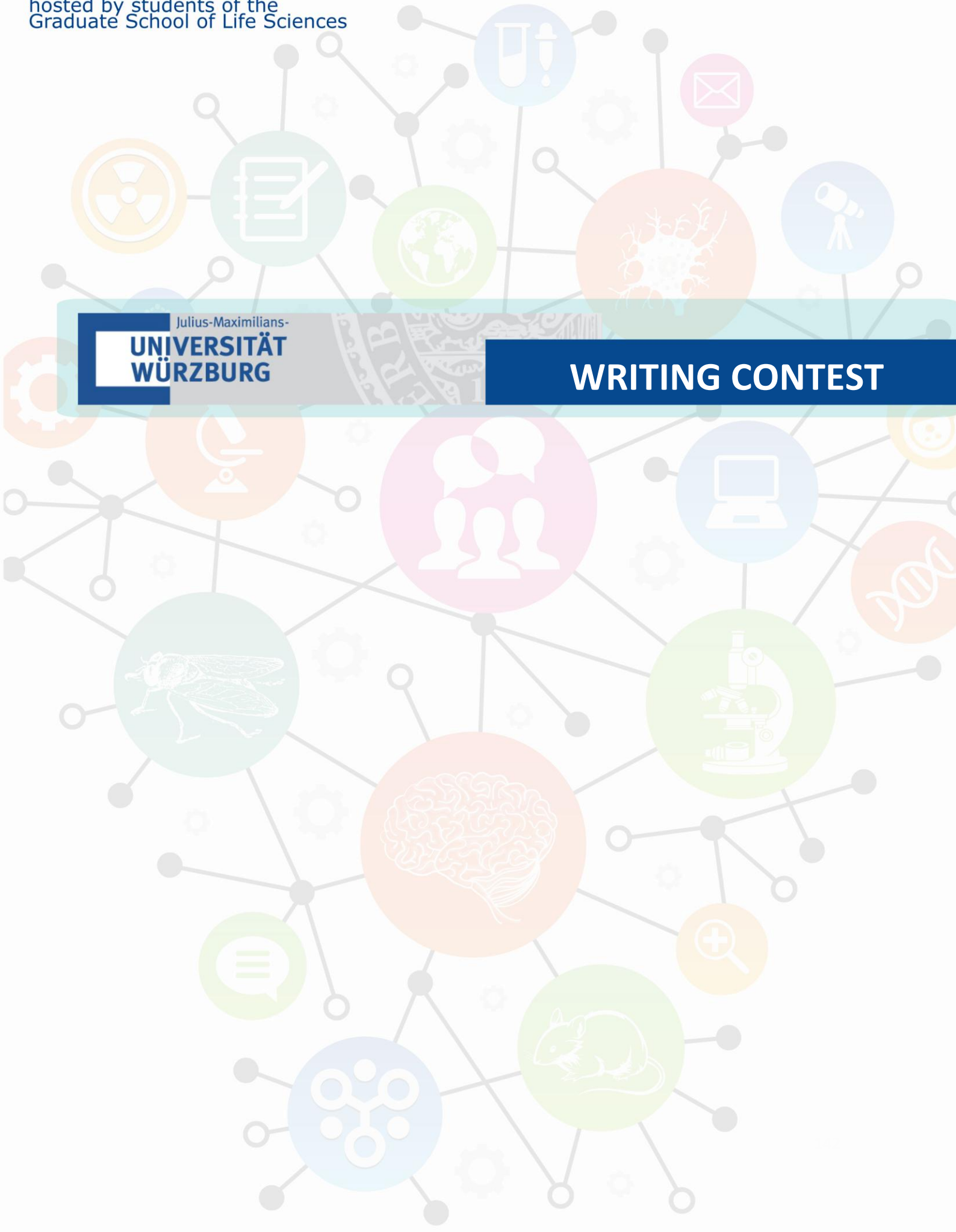
Sahel Shemshadi¹, Annabelle Rosa², Sarah Schäfer², Alma Zerneck-Madsen², Sarah Beck¹

Background Platelets continuously patrol the vasculature, monitor its integrity, and control the trafficking and function of immune cells. These immune-related activities are typically initiated via adhesive membrane receptors (GPIb, GPVI, CLEC-2). GPV, part of the GPIb-V-IX complex, is proteolytically cleaved by thrombin and thereby spatio-temporally controls thrombin activity and fibrin generation. CLEC-2 is critical for blood-lymphatic separation and vascular homeostasis. Mice deficient for GPV and CLEC-2 display altered vascularisation pointing to a loss of vascular barrier function, however how GPV and CLEC-2 synergistically protect from vascular inflammation is not understood. **Aim** To understand how GPV and CLEC-2 synergistically work together to ensure vascular barrier function and how their crosstalk affects (peri-)vascular immune cells. **Methods** Tissue and whole blood flow cytometry was used to characterize immune cell (IC) populations in GPV/CLEC-2 double deficient (DKO), the respective single KOs and wt control animals. This was complemented by IF stainings of IC composition in various organs as well as assays to study edema formation. **Results** Increased endothelial leakage and blood-filling of the peritoneal cavity are observed in *Clec-2^{-/-}/Gp5^{-/-}* mice. Upon macroscopic analysis, *Clec-2^{-/-}/Gp5^{-/-}* adult and old mice exhibited a dramatic inflammation of the lungs and liver; and misconnected blood and lymph vessels in the mesenteries. The infiltration of various immune cells into the lungs and liver generate clusters of immune cells next to the vessels, called Tertiary Lymphoid Structure (TLS). Higher weight of lungs, as well as the enlargement of heart led us to further analysis of edema formation assay, which enlightened the inflammation but not edema driven weight change in the DKO lungs and heart. Flow cytometry highlighted the increased leukocytes in the GPV/CLEC-2 DKO mice including CD4⁺ and CD8⁺ T cells, B cells, neutrophils, and monocytes in the lung; B cells, inflammatory macrophages, and neutrophils, in the heart, and circulating CD4⁺ T cells, Tregs, and cDC1 in the whole blood. Therefore, the increased immune cells, while faced with the growing counts of T regulatory cells, breed a broad immune activation with counter-regulation in the GPV/CLEC-2 DKO mice, resembling a chronic inflammation profile. **Conclusion** DKO mice exhibit IC infiltrations in major organs resembling TLS. Together with HEV formation in the heart, our data point to a chronic inflammatory state in these mice by far exceeding phenotypes of single KOs.

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WRITING CONTEST



Text 1

Just H₂O

Katharina Götz

Rudolf Virchow Center, Research Center for Integrative and Translational Bioimaging, University of Würzburg, Germany | Department of Biotechnology and Biophysics, University of Würzburg, Germany

“Just H₂O”.

That was the dull declaration of my brother when I told him enthusiastically about my current work in the laboratory. “The whole day you are just pouring H₂O back and forth”. Since I’ve never been particularly quick-witted, I couldn’t come up with a good answer.

Performing my next experiments, I took a closer look at the clear and colorless liquid inside the reaction vessels. He was right, looks like just H₂O. For sure, I know that there are also dissolved salts, enzymes and DNA inside and I can do great experiments with it.

Secretly though, I do sometimes have my doubts about that, especially after an unsuccessful outcome. Did I make everything right? Was it the correct DNA concentration? Were the enzymes already degraded? Were there any impurities in my buffer? The next day, I supposedly do everything the same way and then it suddenly works.

Why? I am not sure myself. My brother is not satisfied with that explanation, even though it was always just H₂O.

But in the end, we are all made of just H₂O, too.

Text 2

Think Inside the Box

Shrey Jain

University Hospital Würzburg

I have always believed that if we look closely enough at nature, it has already given us most of the answers. Science, to me, is often the art of noticing them.

My EUREKA moment began with a scientific question: could we use B cells to discover antibodies against intracellular tumour antigens, targets hidden inside cancer cells and therefore difficult to reach? Working on B cell receptor-based hybridoma enrichment showed me that we could. But the real revelation came afterward.

As I began working with human B cells to search for tumour-specific intracellular antigens, I started thinking beyond the experiment. B cells are remarkable. So are T cells. So are NK cells. Yet none of them, alone, seemed sufficient to confront something as adaptable as cancer. And suddenly, cancer began to look less like a problem in a textbook and more like a lesson in life.

We rarely overcome life's greatest challenges alone. Families, communities, friendships, ecosystems - each survives through countless individuals doing different things, compensating for one another's weaknesses and amplifying one another's strengths.

Why should our immune system be any different?

Cancer is a monster born from our own cells. It learns, adapts and finds ways to survive. Perhaps defeating it does not mean finding one perfect weapon, but understanding how the different parts of our immune system can work together in symphony.

That realization changed the question that followed. The goal was no longer simply to find a target, but to uncover vulnerabilities that could engage an entire orchestra of immune cells - each with its own instrument, its own strength, and its own role in creating something no single player could achieve alone.

My EUREKA moment was not discovering that one cell could defeat cancer. It was realizing that nature has never really worked that way. From the smallest cell to the largest ecosystem, life is built on relationships, on different parts doing what they do best, together becoming something greater than any one of them could be alone.

Perhaps that is why I keep looking to nature for answers. It reminds us that the solution does not always lie in finding something new, but in seeing what is already there in a different way.

We are so often told to think outside the box. Perhaps, when it comes to understanding life, we should first learn to think inside the box.

Text 3

The Scientific Chandelier

Duy Thai Duong Le

AG Nagy - Institute of Experimental Biomedicine I



When I first came to Würzburg, my professor introduced me to a chandelier in the RVZ stairway between D15 and D16. When I saw it, my first thoughts was *“Okay, that’s dangerous - like the dangling sword of Damocles”*.

Why would anyone want to put expensive, usable scientific tools on a chandelier, hidden away where most people are probably too busy to stop and appreciate it? Moreover, if those weren’t broken by scientists trying to discover something new, then, it’s a waste if you asked me.

Then, after a year, it hit me: there is some truth to it.

Precision provides the light for science.

Immunoprecipitation (IP) was something I did hundreds of times in another country, and it had always worked perfectly. Yet, for some reasons, it did not work in Germany. The same reagents were used with the same equipment and method, but somehow, the proteins were not detectable in the IP.

After several failed attempts, I finally realized what went wrong. Previously, the buffer I made with those ingredients always showed the optimal pH levels, so I had stopped measuring it. I became careless and thought it would be the similar case here.

Spoiler alert: it wasn’t.

The pH was too high to maintain the protein complex, thus, their interaction was undetectable in my IP.

All in all, science advances through precision. Check everything. Measure everything. And never assume that something is the same just because it worked before. I still have so many things to learn and it has been a fun ride on this PhD journey. I’m looking forward to the following years.

If you’d like to discuss or share what you’ve learned on your own PhD journey, I’d be delighted to meet you at poster #48.

Text 4

When the Experiment Fails, the Visa Should Not Be the Next Thought

Raymond Okokoh

Translational Paediatrics, Kinderklinik, Center for Experimental Molecular Medicine, University Hospital Würzburg

It is Friday afternoon. The experiment has failed again.

The cells that behaved perfectly last month have suddenly decided that differentiation is optional. Thirty days of work have produced a graph that requires considerable imagination to call a result.

Every experimental scientist knows this moment. You question the protocol, the reagents, and briefly yourself. Then you accept a fundamental truth of research: experiments sometimes fail. You redesign and try again.

For many international doctoral researchers, however, another calculation begins:

How many months remain on my contract? What happens to my residence status if my project takes longer than my funding?

Suddenly, a failed experiment has degenerated into an immigration problem.

Research is inherently uncertain because the answer is not known. If the answer were guaranteed, it would not be research. Cells do not differentiate faster because funding is ending. Viruses do not replicate according to grant milestones. **Antibodies occasionally appear to have personal disagreements with PhD students.**

But scientific uncertainty becomes heavier when accompanied by immigration uncertainty. The question can shift from *“What is the best experiment to answer this scientific question?”* to *“What experiment can give me publishable data before my contract ends?”*

Those are not always the same experiment.

International researchers may therefore be running two experiments simultaneously: one asks whether the hypothesis is correct; the other asks whether they can remain long enough in the country to answer it.

We cannot remove uncertainty from science, nor should we. But universities, supervisors and policymakers can reduce unnecessary uncertainty around the scientist through clearer completion-phase residence options, earlier institutional support and better coordination. I believe I am speaking to the future decision/policy makers.

Experiments will fail. Protocols will stop working. Hypotheses will collapse.

Those are problems scientists are trained to solve.

But when the experiment fails, the visa should not be the next thought.

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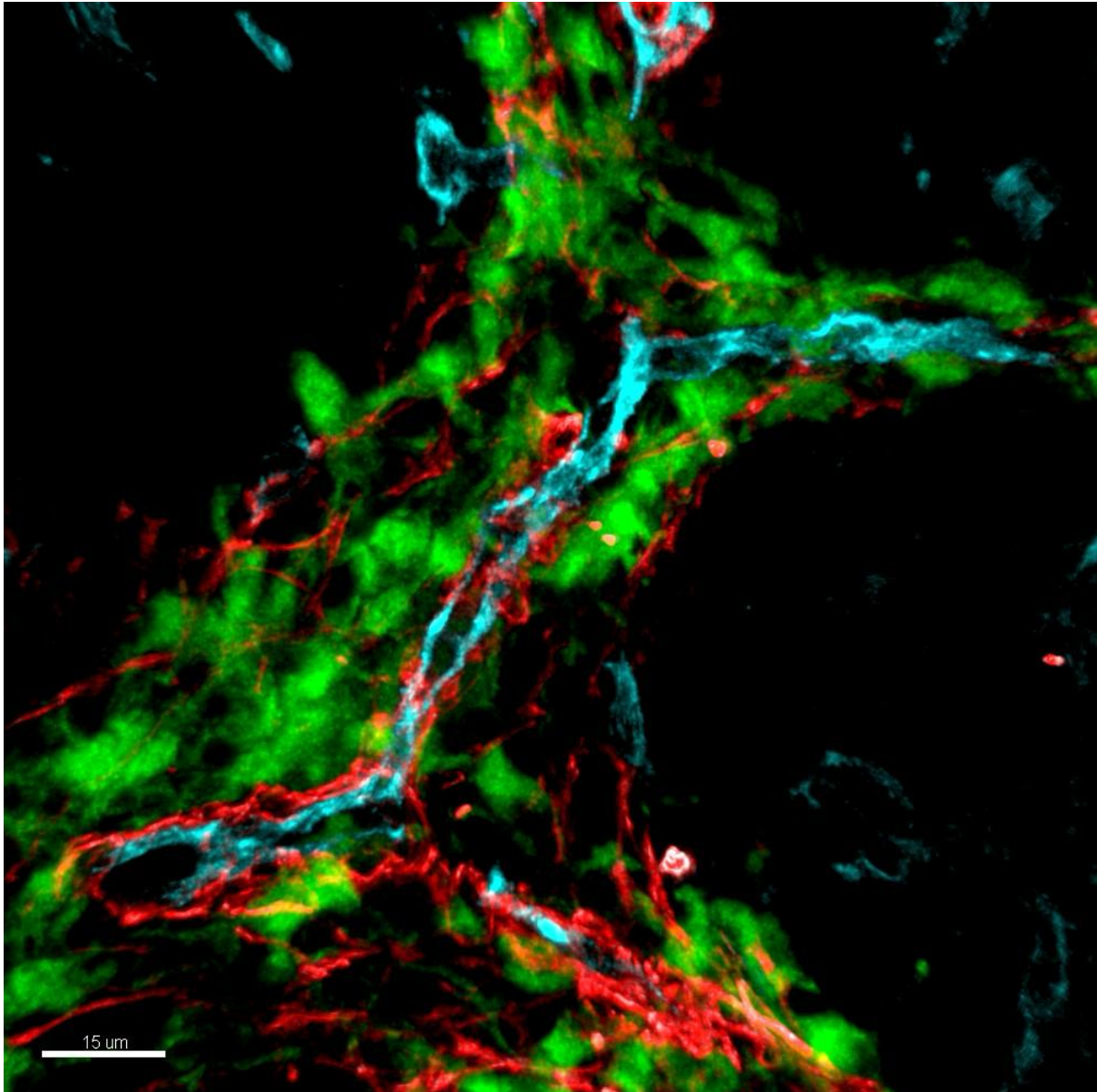
IMAGE CONTEST



Image 1

Dendritic cells surrounding the arteriole in the spleen

Anagha Rajeevalochana



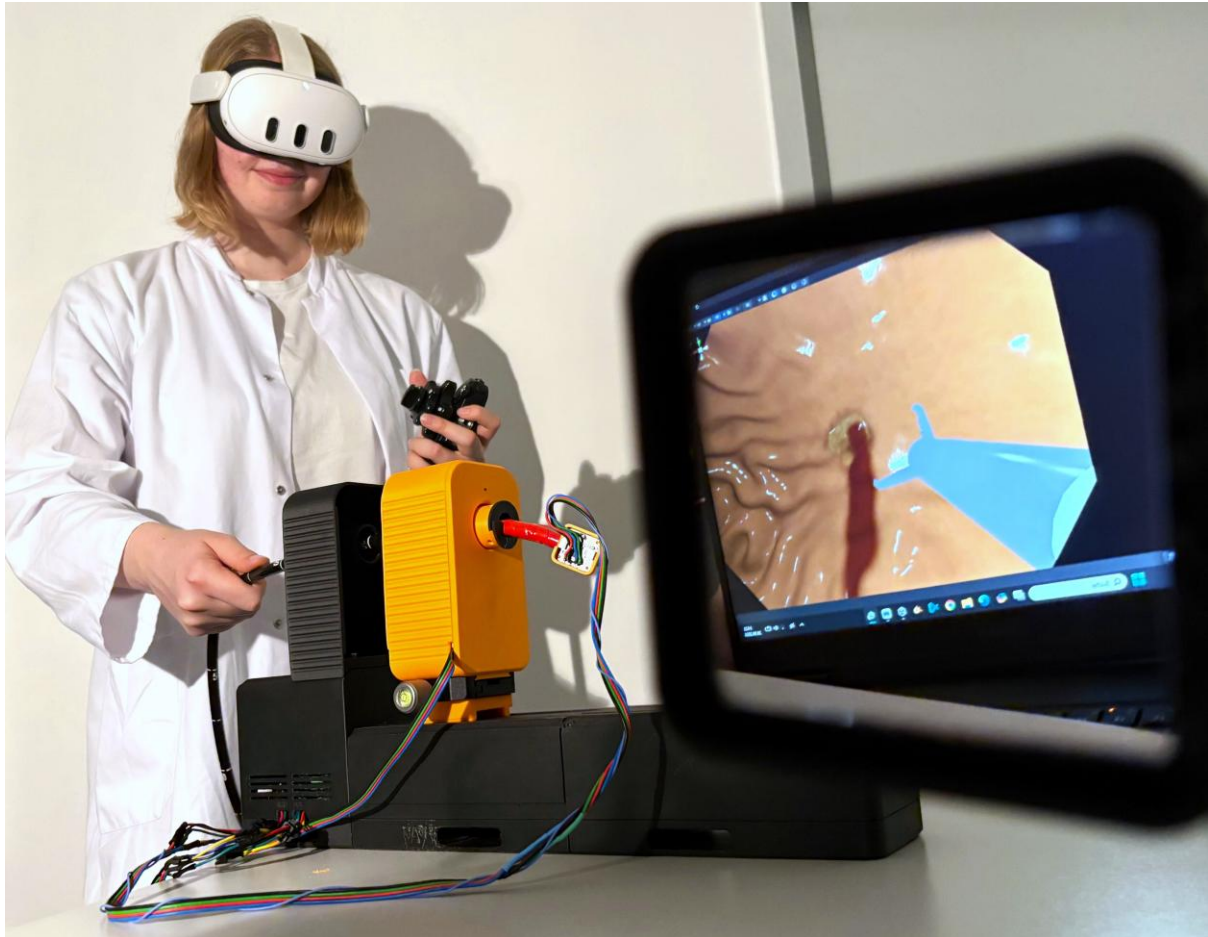
Description- Green- XCR1+ cDC1, Cyan- CD31, Red- aSMA.

Splenic cDC1 migrate from the red pulp to the white pulp by following perivascular tracks surrounding arterioles.

Image 2

Endoscopy – View Through a Looking Glass

Annika Köhler



We developed OPTESK, a virtual reality (VR) training simulator for the endoscopic treatment of upper gastrointestinal bleeding. The endoscope's movements are tracked by sensors and transmitted to the VR simulation. The mirror reflects the endoscopy screen as seen by the user through the VR glasses.

Image 3

Inside Out!!!

Harsha Manjunath

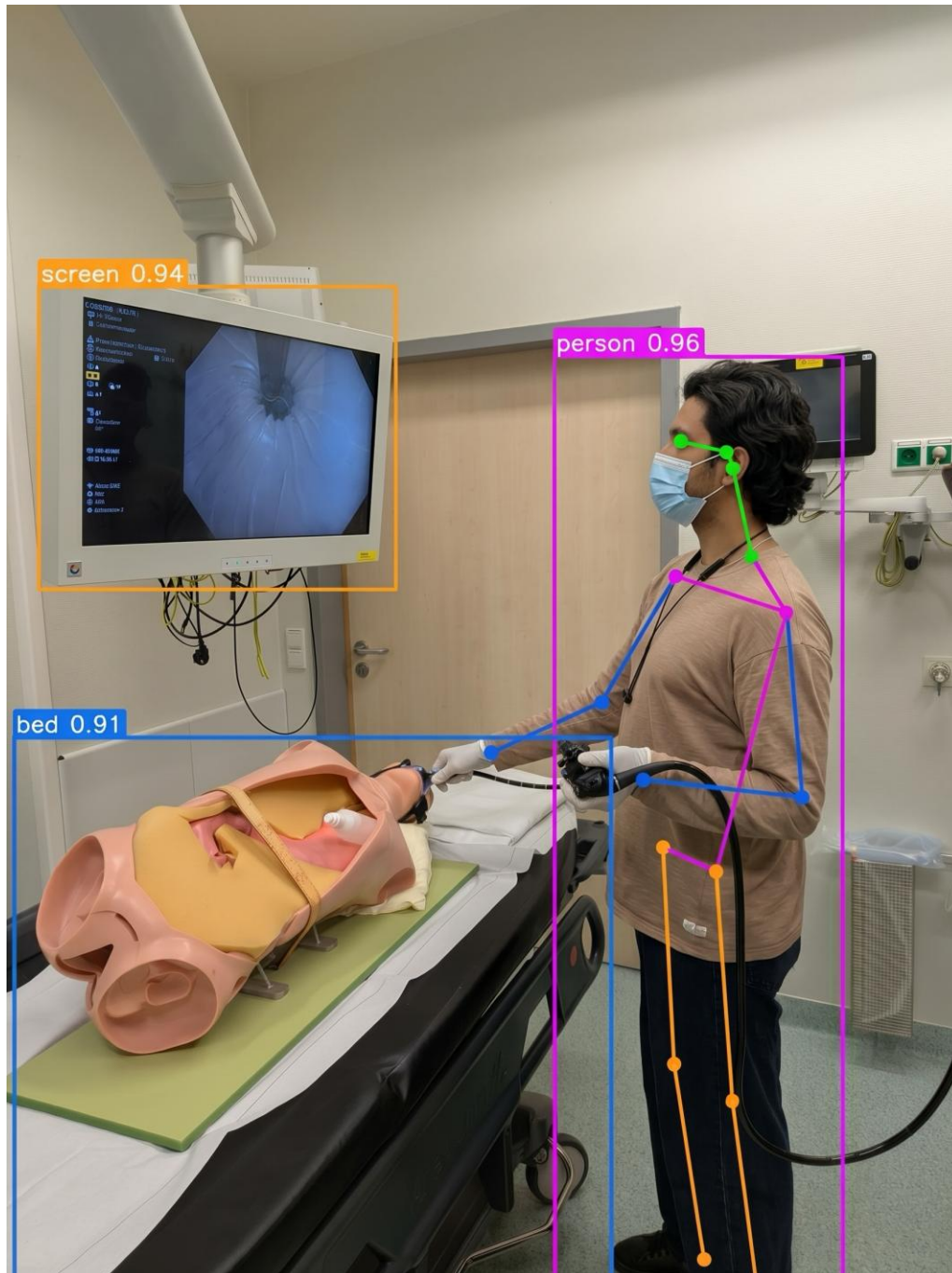


This is my face, rendered as colonic mucosa. SiMucosa, our real-time AI style transfer, was built to make silicone endoscopy phantoms look like living tissue so trainees can practise on something convincing. Pointed at a portrait instead, it does this. The model knows one style and applies it to everything.

Image 4

PostureMeister: AI-driven ergonomics for endoscopy.

Irshath Ali Syed

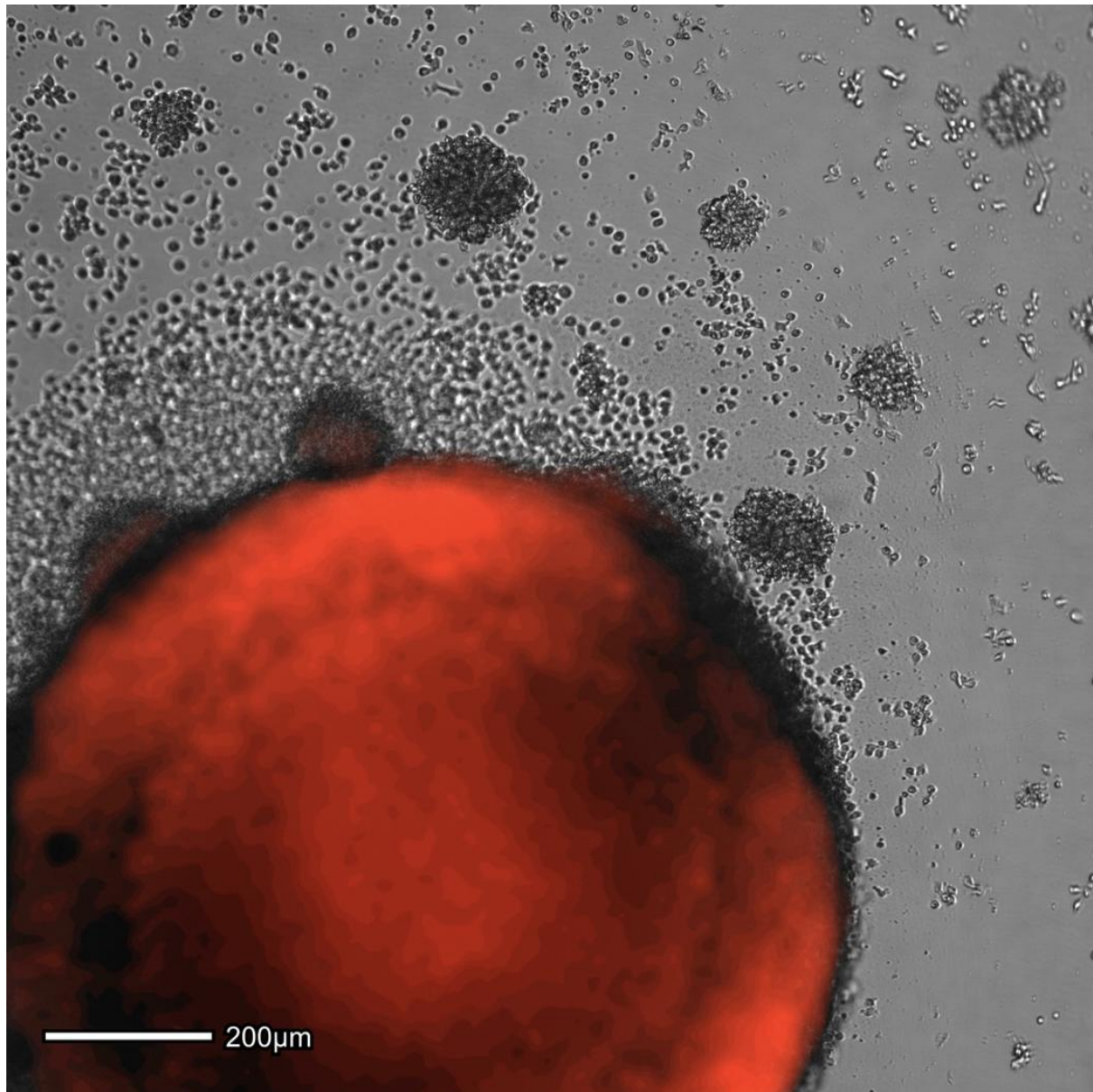


Guidelines for Ergonomic Endoscopy exist only on paper and nothing verifies them in the Endoscopy-room. PostureMeister uses depth cameras and pose estimation to track body keypoints, then computes an optimal monitor and bed height for each individual and compares it against ASGE guideline targets. The goal is healthier endoscopists and longer careers.

Image 5

A Cellular Battle Against Glioblastoma

Maximilian von Bank



GD2-targeted CAR T cells attacking a glioblastoma organoid.

Image 6

We CARE about tu(a)mor ❤️

Sabrina Ciccone

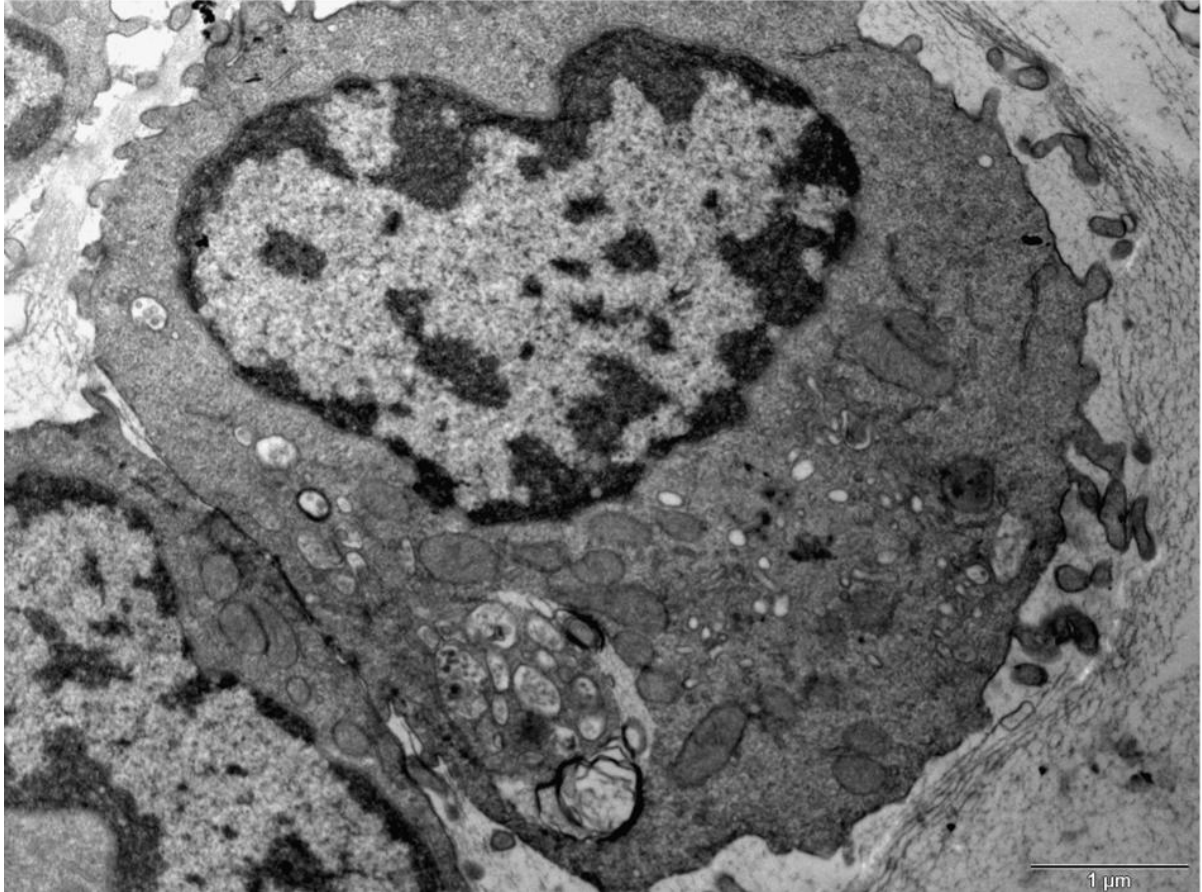


Image 7

“Just don’t...”

William Lokossou Sanvi



I think no description is needed

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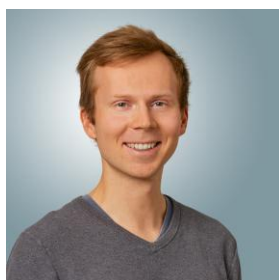
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Thank you!