11th SCRM PhD Students Retreat

Gurten Park

10th September 2024

SCRM
Bern Stem Cell Research and Regenerative Medicine
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CSL Behring

11th SCRM PhD Students Retreat

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10th September 2024

08:30	08:35	Funicular from Wabern to Gurten
08:45	09:00	Welcome Coffee
09:00	09:10	Welcome by Organizing Committee
Morning Session Chairs: Marel Steinfort		
09:10	09:30	Neda Salimi Afjani
09:30	09:50	Filipa Moreira-Silva
09:50	10:10	Wanli Cheng
10:10	10:30	Sarah Peisl
10:30	11:00	**Coffee Break**
11:00	11:20	Siavash Rahimi
11:20	11:40	Haiyan Yue
11:40	12:00	Daniel Batora
12:00	13:00	**Mentor Talk: Dr. Luca Tamò**
13:00	14:00	**Lunch Break**
Afternoon Session Chairs: Francesco Bonollo		
14:00	14:20	Simone Zwicky
14:20	14:40	Andrea Brunello
14:40	15:00	Kimberly Schmied
15:00	15:30	**Coffee Break**
15:30	15:50	Audrey Galé
15:50	16:10	Isabel Schultz-Pernice
16:10	16:40	CSL Behring Presentation
17:00	18:00	**Mentor Talk: Prof. Dr. Alireza Mashaghi**
18:00	18:10	Conclusive Remarks and Thanks from the SCRM Steering Committee

This event was made possible with the generous support of:

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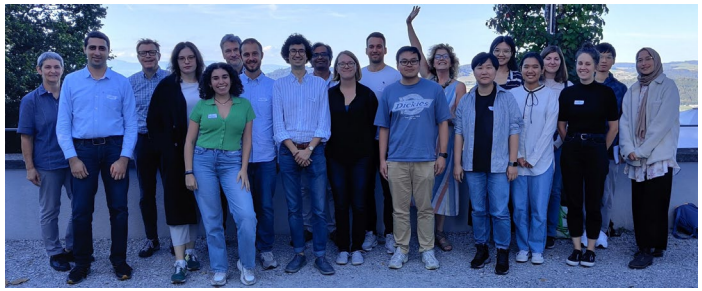
We want to thank you for your support, which made this event possible!

8th Retreat, Gurten Park, 3rd September 2021

9th Retreat, Gurten Park, 2nd September 2022

10th Retreat, Gurten Park, 1st September 2023



SCRM
Bern Stem Cell Research and Regenerative Medicine
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11th Annual PhD Students Retreat

2024 | September 10th
Gurten Park, Bern

Highlights:

- Discuss your ongoing research and future plans
- Career overview
- Learn about the benefits and drawbacks of academic research compared to industry
- Discuss opportunities after your PhD with the specialists

Mentors:

Alireza Mashaghi
Head of the Medical Systems Biophysics & Bioengineering
Leiden University, Netherlands

Luca Tamò
Clinical Operations Program Manager
Global Clinical Operations GCO
Novartis Pharma AG

Register online: www.scrm.unibe.ch
Participation → free of charge
Presentation → 0.5 ECTS

Welcome!

Dear participants,

Welcome to the 11th SCRM PhD Students Retreat! We are happy to continue with you this successful history of PhD students retreats, which started in 2014 and was initiated by our colleague Dr. Luca Tamò, who is also our industry mentor this year!

The day starts with a coffee and a short welcome from the organizing committee. The program will continue with a morning and an afternoon session of PhD project presentations and two coffee breaks for networking and discussions.

We are also excited to attend two interesting keynote lectures, which will be given by this year's mentors **Dr. Luca Tamò**, clinical program manager and global clinical operations GCO at Novartis Pharma AG; and **Prof. Alireza Mashaghi,** head of the Medical Systems Biophysics and Bioengineering at Leiden University, Netherlands. The retreat will be concluded with a special talk from our founding partner **CSL Behring** and some brief remarks by the SCRM Steering Committee.

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

Sincerely yours,

The organizing committee

Ainhoa Asensio Aldave
Francesco Bonollo
Siavash Rahimi
Marel Steinford

Guests

The SCRM Steering Committee:

- Prof. Dr. Andreina Schoeberlein
- PD Dr. Med. Amiq Gazdhar
- Prof. Dr. Volker Enzmann
- Prof. Dr. Benjamin Gantenbein
- Prof. Dr. Marianna Kruithof-de Julio
- Prof. Dr. Paola Luciani
- Prof. Dr. Eliane Müller
- Prof. Dr. Carsten Riether
- Prof. Dr. Deborah Keogh-Stroka

SCRM

The **Stem Cell Research and Regenerative Medicine** (SCRM) Platform is an inter-faculty and inter-institutional research cluster of the University of Bern and the Inselspital, University Hospital Bern. The platform was founded in November 2012 with the aim of facilitating new ideas and skills in translational stem cell research and cell-based therapies to flow seamlessly through the member groups.

The SCRM Platform comprises **over 30 member groups** affiliated with the Medical, Vetsuisse and Phil.-nat. Faculty of the University of Bern and the Inselspital, University Hospital Bern.

The SCRM Platform organizes a number of events every year including a monthly lunch seminar, an annual PhD Students Retreat and an Annual Meeting. To learn more follow us in our official website!

www.scrm.unibe.ch

Isabel Schultz-Pernice

Distinct mechanisms drive tick-borne encephalitis induced cell-death in human neural organoids.

Isabel Schultz-Pernice[1,2,3], Amal Fahmi[1,2,3], Blandina I. Oliveira Esteves[1,2], Teodora David[1,2], Antoinette Golomingi[1,2], Beatrice Zumkehr[2], Selina Steiner[4], Carlos Wotzkow[4], Fabian Blank[4,5], and Marco P. Alves[1,2,6]
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[5]Department of Pulmonary Medicine, Inselspital, Bern University Hospital, Bern, Switzerland
[6]Multidisciplinary Center for Infectious Diseases, University of Bern, Bern, Switzerland

Tick-borne encephalitis (TBE) is a severe neurological disorder caused by infection with tick-borne encephalitis virus (TBEV). Despite the availability of two vaccines, the incidence of TBE has risen over the past decades, becoming a major health concern both in Asia and Europe, including Switzerland. This increase is driven by factors such as changing climatic conditions, evolving human habits, and socioeconomic factors, which influence vector distribution. Despite the urgent need for better understanding and characterization of TBE, the exact pathological mechanisms driving acute neurological manifestations remain poorly understood. Current knowledge primarily relies on rodent model studies, offering limited insights into the neuropathogenesis in the human host.
In this study, we used human neural organoids (hNOs) to explore the susceptibility of human cerebral tissue to infection with three different TBEV strains, including two Swiss tick isolates.
Our findings reveal that hNOs are highly susceptible to infection with all three TBEV strains, as shown by the rapid increase of infectious virus release, viral RNA loads, and frequency of infected cells following virus challenge. Light microscopy analysis showed significant outer morphological changes, such as an increase in hNO surface area and notable cell detachment during infection. Consistently, via flow cytometry, we measured a high level of expression of the apoptotic marker cleaved caspase 3 in infected organoids. Using confocal microscopy imaging, we confirmed prominent tissue damage. While TBEV antigen was preferentially localized in neuron-dense organoid areas, cell type analysis revealed both SOX2 and MAP2 signals decrease over the course of infection, suggesting loss of both neural progenitor cells and neurons. Furthermore, cleaved caspase 3-positive cells were primarily localized in the organoid's ventricular units, indicating apoptosis of neural progenitor cells.
Our findings hint to possible distinct cell death mechanisms affecting neural progenitor cells and neurons during TBEV infection, highlighting the urgent need to elucidate the neuropathological mechanisms of this widespread virus.

Neda Salimi Afjani

Flow culture: An *In Vitro* Model to Investigate the Role of Endothelial Cells Under *In Vivo*-like Flow Conditions.

Neda Salimi Afjani[1,2], Dominik Obrist[3] & Robert Rieben[1]
[1]Department for BioMedical Research, Cardiovascular Disease Program, University of Bern
[2]Graduate School for Cellular and Biomedical Sciences, University of Bern
[3]ARTORG Center for Biomedical Engineering Research, University of Bern

Background: Cardiovascular biologists often face limitations studying blood vessel cells in vitro under diverse flow conditions and with performing all downstream biological experiments. The present 'flow culture' model, featuring a pumpless system with an open circular channel, provides a promising solution. This approach is adaptable, modular, scalable, and suitable for extended experiments.

Method: The validation of this model involved assessments from both physical and biological perspectives. Particle Image Velocimetry (PIV) was used to validate the physical aspects of flow profiles and resultant shear stresses. For biological evaluations, endothelial cells (ECs), either as a monoculture or co-cultured with smooth muscle cells (SMCs), were assessed for cellular alignment using the cytoskeletal marker (F-actin), for their capacity to synthesize the vasodilatory molecule endothelial nitric oxide synthase (eNOS), and the expression of the tight-junction molecule, Pecam-1. Alpha-smooth muscle actin (α-SMA) served as a marker for SMCs. Biological assessments employed immunofluorescent staining and immunoblotting. Individual pigs provided the SMCs (PSMCs) and the aortic ECs (PAECs). A circular open channel, facilitated by a central magnetic cylinder anchored to a ferrous sheet, was created within a glass-bottomed well. In monoculture experiments, ECs were seeded into the channel following a two-step coating process and exposed to varying shear stresses over 48 hours using a rotating tilt mixer (6° inclination). Conversely, co-culture experiments involved seeding both cell types on opposite sides of a pre-coated porous membrane. Following a static phase to enable cellular interactions, the membrane was positioned within a well (secured by a magnetic cylinder) with the PAECs facing the flow and subjected to shear stress levels of 0 and 12 dyn/cm².

Results: PIV analysis revealed unidirectional pulsatile flow within this flow-culture system with generated shear stresses between 0 and 12 dyn/cm². The biological assessments indicated that under both static and flow conditions, ECs developed into a monolayer and exhibited positive expression for Pecam-1, F-actin, and eNOS. Notably, under flow conditions, F-actin filaments displayed increased polymerization and alignment toward the direction of flow. Moreover, ECs demonstrated a statistically significant upregulation of eNOS expression under flow compared to static conditions. In co-cultures, both PAECs and PSMCs formed individual monolayers and demonstrated resilience to 48 hours of high shear stress (12 dyn/cm²).

Conclusions: This well-characterized flow culture model is a reliable system that offers biologists a robust *in vitro* platform to explore scientific inquiries where the influence of blood flow is crucial.

Filipa Moreira-Silva

G9a inhibition by CM-272 mitigates tumour cell survival in advanced prostate cancer.

One of the major challenges in prostate cancer (PCa) is the management of castration resistant disease (CRPC), a highly morbid and lethal stage for which no curative therapy is available yet. Recently, chromatin accessibility has been shown to play a relevant role in advanced PCa stratification, holding promise as a therapeutic target. G9a, a histone methyltransferase responsible for catalyzing the di-methylation of lysine 9 on histone 3 (H3K9me2 modification), plays a crucial role in regulating chromatin architecture. Importantly, G9a has been shown to have a tumorigenic role, including in PCa, prompting the development of different inhibitors over the years. CM-272 is a new and highly selective G9a inhibitor that showed strong anti-tumoral effects in several solid tumors, both *in vitro* and *in vivo*. Specifically, the treatment reduced the global chromatin levels of H3K9me2, decreasing cell viability and proliferation while inducing apoptosis *in vitro*. In *in vivo* models of hematological malignancies, bladder and hepatocellular carcinomas, and melanoma, CM-272 treatment retarded tumor growth and delayed disease progression. We demonstrated previously that G9a inhibition by CM-272 had a strong anti-tumoral effect on multiple PCa cell lines. Herein, we investigated the therapeutic potential of the G9a inhibitor CM-272 *in vitro* and *in vivo* on clinically relevant models of advanced PCa. The effects of CM-272 were evaluated *in vitro* on organoids from patient-derived xenograft (PDX) models, BM18 and LAPC9, representing different disease settings. While both PDX models were derived from PCa bone metastases, BM18 represents an androgen-dependent disease model, while LAPC9 models present an androgen-independent growth typical of the CRPC progression stage. The organoids from both models were treated with CM-272 at multiple concentrations for 48 hours, and cell viability, organoid morphology, and apoptosis were assessed at the endpoint. Additionally, whole-mount immunofluorescence analysis was performed to determine markers of cell proliferation (Ki-67), together with G9a expression and activity. Lastly, to better understand the anti-tumoral properties of CM-272 *in vivo*, BM18 PDXs were administered with CM-272 intraperitoneally for three weeks, measuring tumor growth kinetics, as well as size and weight at the endpoint.
Our results demonstrated that CM-272 significantly reduced BM18 and LAPC9 organoid viability in a dose-dependent manner, affecting multiple morphological features. We determined that the drug IC50 concentration was lower for BM18 PDX-derived organoids (4.5µM) compared to LAPC9 PDX organoids (7.3µM). Importantly, a dose- and time-dependent increase in apoptosis and cell death was depicted by the elevation of pSIVA- and PI-positive cells during the 48-hour treatment window, and this result was complemented by a decrease in Ki-67-positive cells. Importantly, treatment-induced inhibition of G9a activity was shown already at the IC50 concentration. Indeed, we observed a significant decrease in the number of H3K9me2-positive nuclei in treated BM18 and LAPC9 organoids (58% and 50%, respectively), compared with the nuclei positivity detected for the untreated condition (85% in BM18 and 86% in LAPC9). Notably, treatment with CM-272 *in vivo* significantly delayed BM18 PDX tumor growth without relevant toxicity. Hence, the role of G9a in PCa growth was confirmed, highlighting how its targeting by CM-272 holds potential for the management of advanced PCa.

Audrey Galé

Audrey Galé[1], Marta de Menna [1], Jan Schmidt [2], Sabina Rebai[1], Martin Wartenberg[3], Marianna Kruithof-de Julio[1, 4]
[1] Department for Biomedical Research, Urology Research Laboratory, University of Bern, Switzerland
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[4] Department of Urology, University Hospital-Inselspital, Bern, Switzerland

Introduction: Pancreatic ductal adenocarcinomas (PDAC) are highly stromal tumors and elucidating the roles of different tumor microenvironment (TME) components is crucial for better understanding of its heterogeneity and better patient management.

Methods: Using metastasis resections from PDAC patients, we have been testing standard-of-care drugs (SOC) on patient derived organoids (PDOs). From the same source of material, a patient-derived xenograft (PDX) model from a soft tissue metastasis of PDAC was generated. Drug sensitivity of both SOC and exploratory drugs was assessed on organoid drug screenings and ex-vivo tumor slices. To investigate the migration potential of the PDX cells, a model of cell invasion in frequently reported metastatic organs for PDAC is currently being optimized using a transwell system.

Results: PDOs have successfully been generated and expanded in 42% of all cases (n=60). Liver metastasis resections tend to have a better formation and expansion efficiency rate with 75% success rate among all liver metastasis cultured. Overall, 62% of all generated organoids could be screened with SOC drugs. Various organoid morphologies, from hollow to solid, have been observed. Response to drug treatment in the PDX has been assessed using both organoids and ex-vivo tissue slices. Similar sensitivities and resistances have been observed in both set ups, concordant with the patient treatment history as well. Additionally, using an AI platform for drug prediction based on RNA sequencing data, tailored treatment options were investigated. A model for assessing cell migration in various metastatic organs for PDAC is currently investigated. Preliminary results have shown that cells have the potential to migrate and invade superficial layers of healthy tissue. A higher number of invading cells were observed in liver tissue compared to lungs or pancreas.

Conclusion: These data suggest that we have created a PDX model for PDAC that represents the patient's biology and sensitivity to chemotherapies represent a promising tool for better patient management.

Wanli Cheng

Investigating key molecular players in putative stem cell subpopulations of prostate cancer.

Background: Androgen deprivation therapy is the standard treatment for prostate cancer (PCa). Nevertheless, despite initial effectiveness, pre-existing cancer stem cell (CSC) populations invariably lead to incurable castration-resistant prostate cancer (CRPC). CSCs are a subset of cancer cells possessing self-renewal properties, driving tumor progression and regrowth. CD44+ PCa cells exhibit more stemness features and are enriched in tumorigenic and metastatic progenitor cells. Here, we aim to explore different subpopulations in tumors based on levels of CD44 expression and investigate whether they have distinct molecular properties and functional characteristics.

Material and Method: The number of CD44+ cells was evaluated in a tissue microarray from primary prostate cancer patient samples (EMPACT cohort) with clinical follow-up. Flow cytometry sorted the CRPC model LAPC9 tumor into CD44 high (CD44-H) and CD44 low (CD44-L) cells. RNA sequencing was performed on these subpopulations to explore their transcriptomic profiles. CD44 expression was validated at the cDNA and protein levels using qPCR and Western blot (WB) analysis. Sorted cells were maintained as organoids in vitro. In order to trace the dynamics of CD44-H and CD44-L subpopulations in vivo, LAPC9 tumor was labeled by fluorescent and luciferent markers. The CD44 status was determined by flow cytometry when tumor growth.

Results: Patients with elevated expression of CD44 (higher number of CD44+ cells) at the time of surgery exhibited a greater propensity for clinic progression (5-year progression-free survival: 75% vs 95%, P=0.03). Furthermore, CD44-H cell ratio increased in the LAPC9 after castration, indicating that CD44-H cells can survive after treatment. Subsequent WB analysis confirmed that CD44 was highly expressed in sorted CD44-H cells, validating the flow cytometry results. Analysis of the CD44 RNA-seq data showed differential expression of CD44 transcript variants between sorted CD44-H and CD44-L cells. Specifically, CD44v10 (CD44-201) and CD44v7-10 (CD44-209) were notably upregulated. In organoid culture, sorted CD44-H cells displayed enhanced formation, indicating they possessed higher proliferative capacity and clonogenicity than CD44-L cells. Furthermore, when GFP-labeled CD44-H cells and RFP-labeled CD44-L cells were recombined in vivo, CD44-H cells exhibited robust proliferation, while CD44-L cells transitioned to a CD44hi state to facilitate tumor growth. Moreover, those GFP and RFP subpopulations displayed the same CD44hi cell ratio at the endpoint.

Conclusion: Elevated CD44 expression is related to an increased risk in primary PCa. In addition, CD44 high cells exhibit high tumorigenicity in vitro and in vivo and can convert to each other. Moreover, the ratio of CD44-H cells in tumor at endpoint is consistent, despite the starting CD44 expression status. Furthermore, the upregulation of CD44v10 and v7-10 in the CD44-H subpopulation may promote tumor formation and metastasis.

Kimberly Schmied

Advancing Feline Coronavirus Research: An RNA Replicon System and Organoid Models for Inhibitor Testing and Cell Tropism Studies.

Sarah Peisl

The role of biliary microbiota in biliary injury and the development of cholangiopathy.

Background: Cholangiopathies are chronic diseases of the biliary tract that can rapidly progress to liver failure. While some disease triggers are recognized, the exact pathogenesis remains unclear, and no treatment improves transplant-free survival. Emerging evidence suggests there is a distinct microbial composition in bile from cholangiopathy patients compared to healthy controls, suggesting a role of biliary bacteria in disease development. This study hypothesizes that alterations in biliary bacteria trigger sustained cholangiocyte activation and inflammation, contributing to cholangiopathy.

Objective: To investigate the influence of biliary bacteria on the inflammatory response within the biliary tree. The initial aim is to develop a technique to directly access the biliary tree in mice to analyze interactions between bacteria and the bile ducts.

Methods: Surgical bile duct injection was established using 8–14-week-old SPF C57Bl/6J mice, under isoflurane anesthesia and extended-release buprenorphine. The procedure entails a 2 cm subxiphoid midline incision to expose and clamp the common bile duct, catheterization of the gallbladder for injection, followed by catheter removal, cholecystectomy, and clamp release. Methylene blue confirmed the delivery into the liver. The distribution of the injected solvent in was analyzed using IVIS CT scan with Optiprep®, revealing predominant accumulation in the left liver lobe 10 minutes post-injection. Using intravital microscopy (LEICA DIVE 8), the bile ducts of the left liver lobe were visualized *in vivo* at 60µm depth after bile duct injection of FITC dextran.

Results: Bile duct injection and *in vivo* imaging provides detailed and informative insights into biliary dynamics.

Outlook: With the bile duct injection and imaging we will now be able to study the impact of bacteria on bile ducts and surrounding immune niches. This approach offers a innovative methods for future therapeutic investigations aimed at modulating the biliary microbiota to improve outcomes in cholangiopathy patients.

Andrea Brunello

Exploring Prostate Cancer Metabolic States to Predict Patients' Response to Therapies.

Introduction: Prostate cancer (PCa) is the most frequently diagnosed malignancy and the second leading cause of cancer-specific deaths in men in Western countries. Metabolic reprogramming is considered a hallmark of cancer, and different metabolic alterations characterize PCa progression, from the benign prostate to the advanced stages of the disease. The aim of this project is to generate a metabolic Atlas of Prostate Cancer states to improve therapies selection for patients. Specifically, we will employ patient derived xenograft organoids (PDXOs) recapitulating the different stages of the disease. Altering their metabolism with either standard of care (SoC) drugs (i.e. Enzalutamide) and specific metabolic perturbators we aim to obtain different metabolic states of PCa. This Atlas may prove its value in the prediction of personalized therapies to revert the cancer metabolic state back to a normal metabolic state.
Method: Mass spectrometry imaging (MSI) is used to analyze metabolomic data on organoids treated with metabolic perturbators. Specifically, the SpaceM method, developed by Alexandrov group at EMBL, is applied to retrieve spatial metabolomic information on the organoids, for both polar and apolar metabolites. From the organoids treated with metabolic perturbators matching RNA is also collected. To couple the metabolomic and transcriptomic information with functional bioenergetic analysis, Seahorse assays are performed to measure oxygen consumption rate (OCR) and extracellular acidification rate (ECAR). Additionaly, transmission electron microscopy (TEM) analysis is used to analyze mitochondrial morphology.
Results: Preliminary MSI analysis on spheroids proved the feasibility of detecting spatial metabolomic data for both polar and apolar metabolites. Spheroids treated with 2-DG, a glycolysis inhibitor, showed and a suppression in the canonical glycolysis intermediates. Clustering analysis could separate the metabolic states according to the few treatment conditions tested. To speed the MSI analysis with the metabolic perturbators a multiplexing approach was employed. Organoids are labeled with four different live dyes, each corresponding to different treatment. This approach allowed simultaneous recording of four different conditions. Preliminary experiments revealed the feasibility of the multiplexing approach, showing enrichment of expected metabolites, according to the treatment; moreover, no significant metabolic alteration was observed due to the labeling approach. Organoids treated with Apalutamide (androgen receptor inhibitor) and Rapalink-1 (mTOR inhibitor) showed a shift on the metabolic state. In parallel, cell lines with the same treatments showed a reduction in both the oxygen consumption rate (OCR) and extracellular acidification rate (ECAR). Mitochondrial morphology investigation revealed a changes after the treatments; specifically, mitochondria with condensed cristae, which are less active, were observed with Apalutamide and Rapalink-1.
Conclusions: Metabolomic changes can be observed using PCa organoids when treated with SoC or metabolic perturbators. Clustering analysis could separate the treatment conditions, identifying specific metabolic states for each treatment.Moreover, the multiplexing approach proved its feasibility, improving the efficiency of the MSI approach. Metabolic states perturbations correlated with changes in the bioenergetics revealed with the Seahorse. These results were also corroborated by an increase in cristae-condensed type of mitochondria, which are associated with decreased mitochondrial respiration.

Simone Zwicky

The impact of intestinal bacterial translocation on postsurgical infections in patients undergoing pancreatic resection.

S. N. Zwicky[1], D. Spari[1], S. Sundaralingam[1], G. Krutkyte[2], L. Mordasini[1], L. Bally[2], G. Beldi[1]
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Surgical site infections (SSI) are the main cause of mortality in patients undergoing pancreatic surgery. Intraoperative contamination by spillage of intestinal fluid is postulated as a main cause of SSI. However, SSI are also a main complication after surgeries without any manipulation of the intestinal tract. Mainly bacteria of intestinal origin are found within SSI. Endogenous intraoperative bacterial translocation from the intestine to otherwise sterile sites, such as mesenteric lymph nodes (MLN) or blood, has been linked to post-surgical infections and may be an alternative explanation for SSI. However, no study has reliably matched bacteria from SSI with bacteria found in the intestine, MLN, or blood on the strain-level. Therefore, the precise location of SSI-causing bacteria within the intestine, their genetic characteristics, and their route of infection remains unknown. This study aims to determine the incidence of bacterial translocation during pancreatic surgery and its relevance for developing SSI. We aim to demonstrate the intestinal origin of bacteria found in MLN, blood, and SSI by bacterial sequencing and to identify potential genetic predispositions that promote or prevent bacterial translocation. By integrating continuous metabolic and nutritional monitoring, we also aim to decipher interactions between metabolic changes in the host during surgery and intestinal microbiota changes and translocation.
Our project is designed as a prospective, monocentric study including patients undergoing pancreatic resection at the Inselspital, Bern University Hospital. At different time points during surgery, small intestinal specimens, rectal mucosal swabs, MLN, and blood are collected. In the case of SSI, swabs from the infected site are obtained. Samples are subjected to both, metagenomic sequencing and whole genome sequencing of single bacterial colonies.
This study will contribute to a better understanding of perioperative changes in intestinal microbiota composition and its association with bacterial translocation and infectious complications.

Siavash Rahimi

Mechanostructural Signaling in Pemphigus vulgaris upon uncoupling of transadhesion.

Introduction: Pemphigus vulgaris (PV) is a severe blistering skin disease caused by autoantibodies targeting in majority of patients desmoglein 3 (Dsg3) or Dsg3/Dsg1, which are desmosomal adhesion molecules expressed by keratinocytes. The binding of these antibodies uncouples extradesmosomal Dsg3 transadhesion which triggers cellular signaling pathways inducing blister formation in the epidermal stem and progenitor cell compartment.

Methods and Results: To investigate the identified pathogenic signaling molecules responsible for keratinocyte adhesion loss and blister development, we first conducted a thorough systematic review. The collective research on PV signaling indicates that anti-Dsg3 antibodies alter the mechanical, structural and biochemical properties of keratinocytes, highlighting the need to establish a comprehensive signaling network to develop novel applications. Our study utilized scRNA-seq, phosphoproteomics, and live cell phenotyping alongside functional approaches such as keratinocyte dissociation assay (KDA) and immunofluorescence microscopy of 2D human primary epidermal keratinocytes treated with AK23, an experimental pathogenic anti-Dsg3 antibody. scRNA-seq analysis and inferred phosphoproteomics data revealed increased proliferation as confirmed by Ki67 immunofluorescence staining and KDA combined with an inhibitor of proliferation (5-fluorouracil). Additionally, amongst others, gene expression changes related to altered replication and cell cycle progression, Rho GTPase modulation and actomyosin contraction, as well as WNT inhibition, supporting earlier findings. These approaches combined with gene knockout further demonstrated that the loss of adhesion induced by AK23 is associated with pathogenic activation of mechanosensitive ion channels (Piezo1 and TRPV3), shifting cell fate towards proliferation, necessitating transcriptional control. Interestingly, E-cadherin did not play a significant role in this process.

Conclusion: Taken together, our findings suggest that the alteration of keratinocyte mechanical properties by AK23 modulates electrical signaling, shifting the fate of keratinocyte subpopulations from stemness or differentiation towards proliferation, reminiscent of a wound healing phenotype. They also identify extradesmosomal Dsg3 as a mechanical sensor in epithelial tissue providing the basis for multiple pharmacological treatment options in PV patients.

Haiyan Yue

Muscle-Invasive Bladder Cancer: Patient-Derived Organoid-Based Characterization of Disease Features and Therapy Response.

Haiyan Yue1, Martina Radic1, Panagiotis Chouvardas 1,2, Marta de Menna 1,3, George N. Thalmann 2, Marianna Kruithof-de Julio 1,2,3, Bernhard Kiss 2
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The high inter-patient heterogeneity of muscle-invasive bladder cancer (MIBC) is the main reason for the high rates of mortality, recurrence, and treatment failure of these patients. This highlights the need for precise and personalized treatments to improve the prognosis of MIBC patients significantly. Bladder cancer (BLCa) patient-derived organoids (PDOs) have emerged as a promising model for therapeutic assessments since they can recapitulate features of the original tumor, such as cellular phenotype and heterogeneity, offering an ideal platform for precision medicine. In our study, we have confirmed that PDOs faithfully retain the characteristics of parental tissues while exhibiting two out of three distinct morphological types: solid (35%) and mixed (65%), while hollow is not represented. The mixed type accounts for most cases, mirroring the heterogeneous nature of MIBC at the PDO level. Interestingly, despite variations in size and weight, solid-type PDOs maintain constant densities, suggesting the robustness of this model. Additionally, with our PDO-based drug screening pipeline, we have successfully tested both BLCa standard-of-care (SOC) and FDA-approved drugs on MIBC patients. We evaluate drug effectiveness following three parameters: viability, apoptosis, and morphological alterations. The results show that the combination of SOC chemotherapies cisplatin and gemcitabine or MVAC (methotrexate, vinblastine, doxorubicin, and cisplatin) expectedly proved successful. Still, some non-SOC therapies like daunorubicin, doxorubicin, and epirubicin worked surprisingly well, almost comparable to SOC chemotherapies. In summary, we have established a biobank of MIBC patients, revealing variable drug responses among MIBC patients, emphasizing the critical importance of conducting in vitro drug screening as a prerequisite for making well-informed clinical decisions. Future efforts will integrate PDO-based drug screening with genomic, transcriptomic, and epigenetic data from the matched parental BLCa specimens to develop a powerful machine learning-based AI framework to predict drug response, further enhancing precision medicine, and to predict compounds that could reverse the most significant Master Regulators (MRs).

Daniel Batora

From bench to bowl: the next generation of taste enhancers with a global impact.

Unlike the perceived nutritional value, taste is a hedonistic feature of food and is the primary factor in ensuring sustained consumption. Thus, improving the palatability of food while reducing the sugar, fat and the overall caloric content is essential to mitigate the diet-induced burden of non-communicable disease. However, discovery of new molecular mechanisms to improve taste was largely lacking in the last two decades which now even diminishes the sales of previously high-growth sectors such as plant-based meat industry.

The calcium-sensing receptor (CaSR) is a tongue-expressed GPCR which drives the kokumi sensation: the increase of intensity, fullness and continuity of basic tastes. CaSR activators are the major components of yeast extracts which are used as seasonings in major sectors of the food industry.
In an in vitro taste receptor screening platform, we have identified a mechanistically novel class of functional positive allosteric modulators (fPAMs) of the CaSR. Unlike previously described PAMs, our molecules increase the functional response of the CaSR, which enables over 100 % activation when combined with existing commercial products. Interestingly, the fPAM effect happens at a dietarily low and narrow concentration range, above which the molecules show an unfavorable taste profile. We validated the novel mechanism suggested by the in vitro data in human taste trials. A significant improvement of flavor was observed when the modulators were combined with the agonists, and no effect was observed in the absence of agonists.
The modulators are naturally abundant and therefore can be scaled globally. Our data suggests that CaSR signals the consumption of the appropriate dosage of important micronutrients.