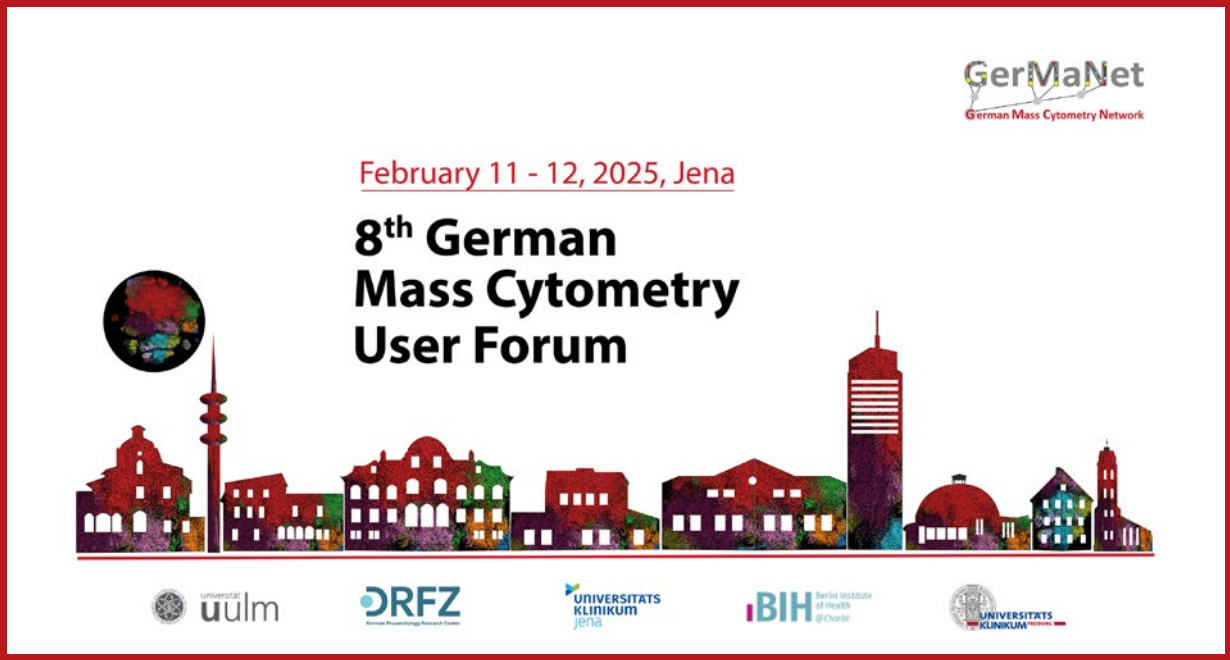
Program overview

8th German Mass Cytometry User Forum



11th – 12th of February 2025

Location
Jena University Hospital
Hörsaal Psychiatrie
Philosophenweg 3
07743 Jena

E-mail
masscytometry@drfz.de

Website
masscytometry.de

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DRFZ German Rheumatology Research Center





Dear friends of mass cytometry,

Welcome to the 8th German Mass Cytometry User Forum, taking place on February 11th and 12th, 2025, in Jena. For many of us, this annual meeting has become more than just a scientific conference – it is a chance to reconnect with familiar faces, share our latest findings, and collectively reflect on the progress we have made as mass cytometrists.

This year's meeting, hosted by Sabine Baumgart and Diana Dudziak from Jena University Hospital, again features a rich and varied program. We are excited to present talks from four distinguished keynote speakers: Carl Lee (University of Oxford), Daniel Bachurski (University of Cologne), Sonia Gavasso (University of Bergen), and Denis Shapiro (Heidelberg University Hospital). Moreover, we will feature the ever-popular "Getting Started" tutorial, and the "News from" sessions, where mass cytometry and imaging mass cytometry sites from Germany and Austria share their latest developments.

The poster session, always a lively part of the forum, will showcase innovative work and provide an excellent opportunity for in-depth discussions. We are pleased to once again recognize outstanding contributions with a poster prize. Additionally, we will award the best abstract-selected talk, both of which are generously sponsored by Denovosoftware.

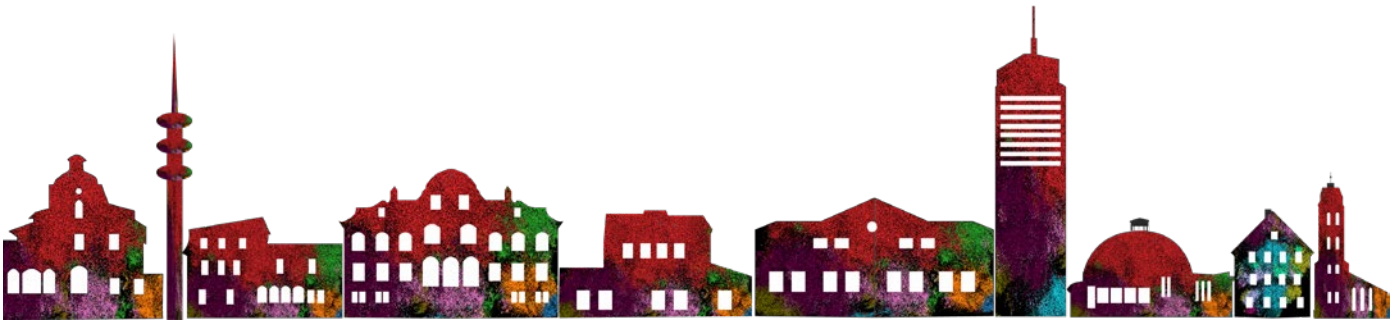
We would like to thank our eight industry partners, some of whom are joining us for the first time. Their support plays a crucial role in making this event possible. We truly appreciate their contribution and look forward to seeing their latest developments and innovations.

Whether this is your first time attending or you have been with us since the early days, we hope you will find these two days in Jena to be enriching, engaging, and enjoyable.

Best wishes,

The organizers of the GMCUF

Sabine Baumgart, Bertram Bengsch, Desiree Kunkel, Henrik Mei, Sarah Warth, Ute Hoffmann, Jacqueline Hirscher, and Axel Schulz



11.02.2025 Tuesday

8:30 am Start of registration

9:00 am Getting startet – Tutorial

Anika Rettig, Berlin, Désirée Kunkel, Berlin, Axel R Schulz, Berlin, Chair: Sarah Warth, Ulm

11:00 am Coffee break

11:30 am - 12:00 pm Welcome

Opening by the meeting organizers

Welcome talk by Diana Dudziak

Universitätsklinik Jena, Germany

Phenotypic characterization of dendritic cell subpopulations in in mouse and human lymphoid and non-lymphoid tissues

12:00 pm - 12:40 Invited talk by Daniel Bachurski, Cologne, Germany

Chairs: Diana Dudziak & Sabine Baumgart

Tellurium-based labeling of the extracellular vesicle proteome (TeLEV) enables high-dimensional analyses and nanometer-resolution imaging of EV recipients

12:40 pm – 1:10 pm Techno bites

Chairs: Sarah Warth & Axel Schulz

BlueCatBio, Cell Signaling Technologies, Ionpath, Parhelia Biosciences, Polaris Biology, Scidentify

1:10 pm – 2:30 pm Lunch break & networking

2:30 pm – 3:45 pm Suspension MC session

Chairs: Henrik Mei & Axel Schulz

Invited Talk by Sonia Gavasso, Bergen, Norway

Immune signature changes in blood of MS patients undergoing aHSCT

Short Talk by Mehmet Serdar Koca, Granada, Spain

CyTOF harmonization for multicenter immune monitoring studies

Short Talk by Lucas Arendholz, Berlin, Germany

High-parameter immunophenotyping using cytometry by time of flight (CyTOF) and unbiased bioinformatical analysis identifies biomarkers of Post-Covid Syndrome ME/CFS and of response to immunoadsorption therapy

Short talk by Jonas Haugsøen, Bergen, Norway

Immune Reconstitution After Hematopoietic Stem Cell Transplantation in Multiple Sclerosis

3:45 pm - 4:15 pm Coffee break

4:15 pm - 6:00 pm News from … Part 1

Chairs: Claudia Peitzsch & Sarah Warth

News from Jena: Christian Puta

What is the impact of acute anaerobic exercise on immune cells in individuals with and without B cell depletion in the context of post-viral infection syndrome? – A case study

News from Berlin (BIH): Jennifer von Schlichting

Putting TeMals to work: Multiplexing of Matrigel embedded, patient-derived organoids using thiol-reactive barcodes

News from Leipzig: Sergio Gomez Olarte

Applications of CyTOF-based immunophenotyping: a case example of chemical mixture effects

News from Ulm: Simona Ursu

Start with CyTOF: Step by step to success from the perspective of a core facility

News from Cologne: Patrick Hölker

News from Cologne – Expanding high-dimensional analyses of aggressive lymphoma, the tumor microenvironment, and extracellular vesicles

News from Berlin (DRFZ): Adrian Barreno-Sanchez

A Mass Cytometry Workflow for Ex-Vivo Phosphorylation and Mucosal Immune Profiling in Systemic Lupus Erythematosusbstract

News from Freiburg: Emilia Schlaak

I will discuss data from translational work that identifies key responding immune populations in patients exposed to checkpoint blockade in HCC.

6:00 pm – 10:00 pm Poster session & dinner

12.02.2025 Wednesday

9:00 am – 9:30 am Product feature talk Standard BioTools

Chairs: Sabine Baumgart & Claudia Peitzsch

Benjamin Ehret, Data Scientist, Navignostics AG

Enhancing IMC through AI-based information transfer between antibody panels

9:30 am – 10:45 am Imaging MC and Data Session

Chairs: Bertram Bengsch & Desiree Kunkel

Invited Talk by Carl Lee, Oxford, UK

Advancing Spatial Biology: Unlocking Clinical Potential with Spatial Proteomics

Short Talk by Huck Adrian, Berlin, Germany,

Fatty acid metabolism-dependent polarization of tumor associated macrophages

Short Talk by Ira Godbole, Freiburg, Germany

Deep profiling of the tumor immune microenvironment for personalized treatment for biliary tract cancer

Short talk by Matthieu van Tilbeurgh, Fontenay-aux-Roses, France

Role of antigen bio-distribution and persistence in early and long-term immune responses to the yellow fever vaccine in non-human primates

10:45 am – 11:30 am Coffee break

11:30 am – 1:00 pm News from … Part 2

Chairs: Sabine Baumgart & Lena Müller

News from Wien: Klára Brožová

Spatial proteomics to reveal intratumoral heterogeneity in breast cancer subtypes induced by the tumor microenvironment

News from Dresden: Ezgi Senoglu

Epi-CyTOF-based investigation of the epigenetic state of the human developing neocortex

News from Heidelberg: Felix Hartmann

Spatial quantification of cellular metabolism identifies metabolic niches predictive of response to immune checkpoint inhibition in metastatic melanoma patients

News from Berlin (MPIMG): Anika Rettig

Quantifying IMC data analysis step by step: a comparative evaluation of MICCRA and traditionally used approaches

News from Erlangen: Aleix Rius Rigau

Imaging mass cytometry-based characterisation of fibroblast subsets and their cellular niches in systemic sclerosis

News from Freiburg II: Florian Ingelfinger

CytoVI: Deep generative modeling of cytometry data across technologies

1:00 pm – 2:00 pm Lunch break & networking

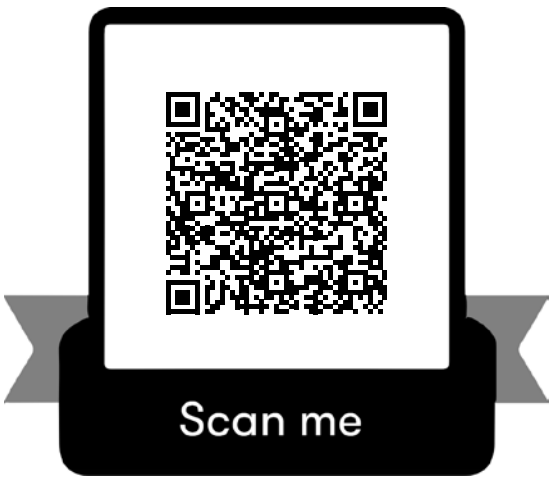
2:00 pm – 2:40 pm Invited talk by Denis Schapiro, Heidelberg, Germany

Chair: Bertram Bengsch & Henrik Mei

From oncology to cardiology: Spatial omics technologies for topographic biomarker discovery

2:40 pm – 3:00 pm Farewell & Award Ceremony

Survey for the 8th GMCUF



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Tuesday, 11th of February 2025

9:00 am – 11:00 am Tutorial: Getting Started

Anika Rettig

Max-Planck-Institut für Molekulare Genetik, Berlin, Germany

Désirée Kunkel

BIH Cytometry Core Facility, Berlin Institute of Health (BIH) at Charite – Universitätsmedizin Berlin, Berlin, Germany

Axel R Schulz

Mass Cytometry Core Facility, German Rheumatism Research Center berlin, a Leibniz Institute, Berlin, Germany

Chair: Sarah Warth, Core Facility Cytometry, Ulm University, Ulm, Germany

Our introduction to mass cytometry ensures that everyone is at the same level when talking about this technology. Four experts from the field tell you how mass cytometry works and how it can be used to examine cell suspensions and tissue sections. We will guide you through typical experimental workflows and share our experience with important aspects in the application of mass cytometry, such as metal conjugation, sample barcoding, spillover compensation and batch normalization. You will also learn about the advantages of Imaging Mass Cytometry (IMC) and how to establish a multiplexed antibody panel for it. This is complemented by an introduction to current concepts of data analysis, both for imaging and suspension mass cytometry. Following the introductory talks there will also be time to discuss individual questions concerning mass cytometry and its application.

11:30 am – 12:00 pm Welcome

Welcome by: Sabine Baumgart, Bertram Bengsch, Desiree Kunkel, Henrik Mei, Axel Schulz, Sarah Warth

Chairs: Diana Dudziak & Sabine Baumgart

Welcome talk by Diana Dudziak

Institute of Immunology, Jena University Hospital, Jena, Germany

Phenotypic characterization of dendritic cell subpopulations in in mouse and human lymphoid and non-lymphoid tissues

The main research focus of the Dudziak lab is the understanding of ontogeny, antigen presentation of primary dendritic cell (DC) subpopulations in mice and men and their function in activation of primary T cell immune responses. In her talk, Diana Dudziak will introduce the world of DC subpopulations in mice and men. She will summarize the function of DC subsets in the induction of T cell responses. Thereafter, she will present the latest data of the lab on DCs in lymphoid and non-lymphoid tissues and the influence of the tissue microenvironment in DC functionality.

12:00 pm - 12:40 Invited talk by Daniel Bachurski, Cologne, Germany

Chairs: Diana Dudziak & Sabine Baumgart

Tellurium-based labeling of the extracellular vesicle proteome (TeLEV) enables high-dimensional analyses and nanometer-resolution imaging of EV recipients

Extracellular vesicles (EVs) are key mediators of intercellular communication within the tumor microenvironment and play essential roles in immunomodulation. However, single-cell (sc) resolution of EV uptake and associated immune effects remains elusive due to methodological constraints.

Here, we introduce TeLEV, a metabolic mass-tag labeling approach employing monoisotopic L-Tellurienylalanine (TePhe) for labeling cellular secretomes. By integrating (imaging) mass cytometry and nanoSIMS, TeLEV captures EV and soluble protein uptake in 14M immune cells, enabling sc-level analyses of dose dependence, time kinetics, and fraction-specific uptake of size-exclusion chromatography fractions. We map these interactions in 50 immune cell populations, examining EVs derived from six primary, treatment-naïve chronic lymphocytic leukemia (CLL) samples and nine cell lines, then validate the findings in 6M single cells from 33 additional CLL samples. Our results reveal that primary CLL-TeLEVs preferentially target monocyte subsets and myeloid dendritic cells in a CD11c- and BTK-dependent manner, altering T cell effector memory and NK cell phenotypes. Moreover, primary CLL and Ramos cell line TeLEVs induce a unique CD25, IL-3R, and IL-7R expression profile in myeloid cells, underscoring the distinctive immunomodulatory function of malignant B cell-derived EVs. Notably, CLL-TeLEVs induce BCL-2 expression and facilitate myeloid cell selection within the CLL TME. Finally, integrative sc multi-omics (CITE-seq and CyTOF) corroborates this EV-mediated selection mechanism.

In sum, the TeLEV approach enables comprehensive sc investigations of EV uptake and function in primary samples and cell lines. Leveraging TeLEV, we identify a novel EV-driven immune selection process in CLL, emphasizing EVs as global modulators of immune cell communication.

12:40 pm Techno Bites

Chairs: Sarah Warth & Axel Schulz

BlueCatBio, Cell Signaling Technologies, Ionpath, Parhelia Biosciences, Polaris Biology, Scidentify

1:10 pm – 2:30 pm Lunch break & networking

2:30 pm – 3:45 pm Suspension MC Session

Chairs: Henrik Mei & Axel Schulz

Invited Talk by Sonia Gavasso

NeuroSysMed, a center for clinical trials in neurology and University of Bergen, Faculty of Medicine, Department of Clinical Medicine & Haukeland University Hospital, Bergen, Norway

Immune signature changes in blood of MS patients undergoing aHSCT

I will present and discuss immune signature changes in blood of relapsing-remitting multiple sclerosis patients undergoing autologous hematopoietic stem cell transplantation (aHSCT), give a brief overview of the rational of aHSCT in autoimmune diseases and a short summary of what we think we know and observe today. I will present our suspension mass cytometry set-up and running protocol for collecting over 200mill events over a short time. Finally, I will present the most interesting results in our new data and propose possible mechanisms of aHSCT.

Short Talk by Mehmet Serdar Koca

Pfizer-University of Granada-Junta de Andalucía Centre for Genomics and Oncological Research (GENYO), Granada, Spain

CyTOF harmonization for multicenter immune monitoring studies

Background: 3TR is a multicenter consortium studying common molecular signatures across 7 chronic diseases. Among various OMICs technologies, cytomics data will be obtained and whole blood samples will be analyzed using mass cytometry. Due to high numbers of individuals (>1000) and limited CyTOF throughput, various instruments need to be involved.

Objectives: Optimize samples acquisition protocol and verify if multiple CyTOF instruments can be used.

Methods: For acquisition solution selection, identical aliquots of blood samples were acquired using CAS, CASPLUS, and water. FlowSOM and PCA analysis was performed. To study spillover matrices stability, 12-plex stained compensation beads were analyzed in fresh or after freezing for up to 3 months. To test multiple CyTOF instruments, barcoded blood from 3 donors was stained (22-plex panel), aliquoted and frozen. Aliquots were acquired using 4 HELIOS and 1 CyTOF XT. Coefficient of variation (CV) was calculated for cell frequencies and median signal intensities.

Results: Increasing background was detected using CAS. Water introduced artefacts during clustering. CASPLUS presented the highest sample stability, if acquired in aliquots for no longer than 1h each. Spillover compensation was consistent during 3 different experiments up to 3 months. Center-based effect was reduced with bead normalization, however CVs >20% were detected in some populations. CytoNorm normalization reduced center enrichment in the clustering analysis, additionally yielding CVs <20%.

Conclusions: Multicenter CyTOF experiments are feasible, however special sample acquisition protocol and additional normalization steps are needed to align the centers.

Short Talk by Lucas Arendholz

Berlin Institute of Health (BIH), Charité – Universitätsmedizin Berlin, Berlin, Germany

High-parameter immunophenotyping using cytometry by time of flight (CyTOF) and unbiased bioinformatical analysis identifies biomarkers of Post-Covid Syndrome ME/CFS and of response to immunoadsorption therapy

COVID-19 infections can lead to symptoms of chronic fatigue and exertion intolerance, persisting for months and even years post-infection – known as Post-Covid Syndrome (PCS) or Long-Covid. The estimated incidence is about 400.000 new cases in Germany every year and no prescribable therapies exist. The heterogeneous clinical appearance points towards multiple etiologies. A subset of patients develops myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) a severe postinfectious sequela of various infections. Clinical studies provided evidence for a role of autoantibodies in ME/CFS. Recent reports connect severe COVID-19 and PCS with an aberrant immune response with some patients developing autoantibodies. These might benefit from IG removal (immunoadsorption, IA), but biomarkers for patient stratification and mechanistic insights are lacking.

Cytometry by time of flight (CyTOF) was used to profile leukocytes in blood samples from PCS-ME/CFS patients (PCS patients meeting ME/CFS criteria) pre- and post-treatment and from age- and sex-matched controls. PCS-ME/CFS patients divided into IA-responder and non-responder. IA-responders showed heightened B cell activation across the entire B cell compartment, whereas non-responders did not, indicating a potential biomarker for PCS-ME/CFS and autoantibody-targeting treatment. This normalized after IA-treatment, suggesting a causal link.

This study presents those B cell alterations as potential biomarkers for PCS-ME/CFS and autoantibody-targeting treatment. Currently, we perform single-cell transcriptome, plasma proteomics and mechanistic *in vitro* analyses to understand which factors might drive the dysregulation of the B cell compartment and its contribution to PCS development. However, the study also highlights the complexity of PCS and the need to identify biomarkers predicting therapy response.

Short talk by Jonas Haugsøen

Neuro-SysMed, Department of Neurology, Haukeland University Hospital and Department of Clinical Medicine, University of Bergen, Norway

Immune Reconstitution After Hematopoietic Stem Cell Transplantation in Multiple Sclerosis

Patients with relapsing-remitting multiple sclerosis (RRMS) may experience persistent disease activity despite treatment with disease-modifying therapies (DMTs) or present with an aggressive, rapidly progressing disease. For these patients, autologous hematopoietic stem cell transplantation (aHSCT) is an alternative treatment option. This procedure has demonstrated effectiveness in halting inflammatory disease progression, achieving long-term disease remission, and restoring immune tolerance by eliminating autoreactive immune cells. However, the mechanisms underlying its efficacy remain incompletely understood.

This study explores how G-CSF mobilization, immunoablation with cyclophosphamide and anti-thymocyte globulin, and subsequent immune reconstitution affect the immune system in RRMS patients undergoing aHSCT. Data were sourced from Arm A of the RAM-MS clinical trial, which compares aHSCT to alemtuzumab, cladribine, or ocrelizumab in RRMS patients. Over 100 million single cells from stabilized whole blood of 25 patients were analyzed using mass cytometry (CyTOF) at several time points – on inclusion and at 3, 6, 12, and 24 months following aHSCT.

Following aHSCT, naïve CD4 and CD8 T cells and memory B cells were nearly entirely depleted, while CD4 and CD8 memory T cells and naïve B cells were rapidly reconstituted. Some of these changes persisted over time. Functional alterations in chemokine and adhesion markers were also observed, suggesting profound immunological reprogramming.

This study provides valuable insights into the dynamic immune changes following aHSCT and enhances our understanding of its mechanisms in treating RRMS, offering a potential for refining future therapeutic approaches.

4:15 pm – 6:00 pm News from … Part I

Chairs: Claudia Peitzsch & Sarah Warth

News from Jena: Christian Puta

Department of Sports Medicine and Health Promotion, Friedrich-Schiller-University Jena, Jena, Germany
Department for Internal Medicine IV (Gastroenterology, Hepatology and Infectious Diseases), Jena University Hospital, Jena, Germany

Center for Sepsis Control and Care (CSCC), Jena University Hospital/Friedrich-Schiller-University Jena, Jena, Germany

What is the impact of acute anaerobic exercise on immune cells in individuals with and without B cell depletion in the context of post-viral infection syndrome? – A case study

Patients with post-acute infections syndromes (PAIS) suffer from a high degree of fatigue and exercise intolerance including post-exertional malaise (PEM). In healthy individuals and even cancer patients, physical exercise has been found to have a positive effect on health-related outcomes caused by immune cell modulation but little is known about the acute response to an exercise stimulus in PAIS patients. The aim of our study was to analyze immune cell modulation in PAIS patients performing a 1-minute anaerobic exercise test. Here, we present a special case of a young female athlete diagnosed with post-acute infection syndrome and treated with Rituximab for depleting B cells.

Our case study focused on a 20-year-old female patient with PAIS, who was treated with Rituximab for a chronic replicative Epstein-Barr virus infection. Peripheral blood was drawn on two time points: 6 months (T1) and 20 months (T2) after receiving the last dose of Rituximab, and additionally two sex/aged matched controls. Samples were collected before and after a 1-min sit-to-stand test at the following time points: base line (BL), post 5, 10, 15, 30, 45 min (P5, P10, P15, P30, P45). Three more sex/age-matched controls were added for the baseline data. Leukocyte subsets were profiled using MAXPAR DIPA assay and Cytometry by time of flight (CyTOF). Data analysis was manually performed using FlowJo v10.10.0.

Data from our PAIS patient show a sustained B cell depletion six months after the last dose of Rituximab (T1) and a plasma blast (PB) frequency higher than in the control group (N = 5). Both subsets were recovered to the normal level in the follow-up sample (T2). However, memory B cells were persistently decreased at T1 and T2. For the T cell subsets, the CD4/CD8 T cell ratio was lower at T1. In addition, persistent imbalance of Th1/Th2 ratio towards Th1 and persistent higher frequencies of CD8+ terminally differentiated effecter memory (TEMRA) T cells were observed at T1 and T2. In depth analysis revealed a special phenotype of CD8+ TEMRA cells: CD8+CCR7–CD45RA+CD28–CD57+CD56+CXCR3+ indicating an acquired natural killer (NK) cell trait. We found that the anaerobic exercise induced the following changes in the immune profile: I) a significant mobilization of absolute numbers of leukocytes immediately after exercise at P5 with a less slope of decline up to P45 in the patient samples compared to the control group (N = 2), II) an about 2-fold increase of NK cell frequencies referred to total CD45 at P5 followed by a reduction below the BL values in the patient and control samples, respectively, iii) the observed modulation was mostly prominent in the CD56+CD16+ NK cell subset – a phenomenon that has been described before in healthy individuals. Interestingly, we also observed a phenotype linked to myeloid derived suppressor cells (MDSC) exclusively after B cell normalization at P15, P30, P45, a phenotype that is associated to severely diseased COVID-19 patients.

This case study highlights the impact of anaerobic exercise on immune cell dynamics in a young female athlete diagnosed with PAIS and treated with Rituximab. The small stimulus induced changes in the immune cell profile including an immediate and transient mobilization of leukocytes and NK cells shortly after starting the exercise. The emerging of specific immune cells such as MDSC points to the complex nature of immune responses in PAIS patients and underscores the importance for further research to verify these observations.

News from Berlin (BIH): Jennifer von Schlichting

Charité – Universitätsmedizin Berlin, Germany

Putting TeMals to work: Multiplexing of Matrigel embedded, patient-derived organoids using thiol-reactive barcodes

Multiplexing in mass cytometry eliminates the confounding effects of technical variabilities in staining, processing, and data collection when comparing multiple samples. The most common multiplexing workflow is based on commercially available, amino-reactive palladium based barcodes. While this method works very well for a multitude of fixed cell types, it poses some challenges for other applications such as 3D cell culture models. These culture models involve cells that are embedded in protein rich, basement membrane like gels (e.g. Matrigel) in low cell numbers. Therefore, a barcoding workflow that enables sample pooling at the earliest possible stage and possibly allows for in situ-barcoding is desirable. A new kind of thiol-reactive, tellurium based barcodes (TeMals) has been introduced and proven suitable for 3D organoid culture. Here, we present our implementation of a TeMal based barcoding approach for patient-derived colorectal cancer organoids, including downstream debarcoding and sample analysis.

News from Leipzig: Sergio Gomez Olarte

Department of Environmental Immunology, Helmholtz Centre for Environmental Research – UFZ and Leipzig CyTOF Team at UFZ, Leipzig, Germany

Applications of CyTOF-based immunophenotyping: a case example of chemical mixture effects

Introduction: Modern populations are daily exposed to complex chemical mixtures. Per- and polyfluoroalkyl substances (PFAS) are of public health concern because they bioaccumulate and adversely affect the immune system. Nonetheless, most studies have addressed PFOS and PFOA immunotoxicity, while neglecting the potential impact of other PFAS and their mixtures. Here, we assess whether a mix of PFAS at concentrations detected in European populations modifies the immunophenotyping of human peripheral blood mononuclear cells (PBMCs) by using CyTOF.

Methods: PBMCs were isolated from 6 healthy donors and cultured with a PFAS mixture at 1x, 100x, and 1000x the human blood concentration for 24 h. The mixture was dissolved in DMSO and contained 6 different PFAS (PFOS, PFOA, PFHxS, PFNA, PFDA, and PFUnDA) with 1x concentrations ranging from 0.19 to 22.34 ng/mL. After exposure, 1.0×106 cells/mL were harvested, stained with the Maxpar Direct Immune Profiling Panel, and acquired in a Helios System.

Results: tSNE high-dimensional reduction and clustering analysis revealed that PFAS-1000x induced marked changes in the expression of cell surface markers among 33 immune subpopulations without affecting their viability. The most susceptible subsets were B and T lymphocytes. Manual gating showed a concentration-dependent decrease in the naïve/memory B cell ratio. Likewise, the ratio of central/effector memory CD4+ T cells exposed to PFAS-100x was significantly higher as compared to the DMSO control.

Conclusions: This preliminary data indicates that the tested PFAS mixture alters the immunophenotyping of PBMCs following acute exposure *in vitro*. Further experiments with greater sample sizes and functional assays are ongoing.

News from Ulm: Simona Ursu

Core Facility Cytometry, Medical Faculty, Ulm University, Ulm, Germany

Start with CyTOF: Step by step to success from the perspective of a core facility

Starting a CyTOF study is exciting but sometimes also challenging. We are presenting an example of the work flow from our core point of view: from the first discussion, to the first tests, towards the actual samples acquiring and workflow documentation. We will also touch base about some issues we encountered in the hope that other core facilities will benefit from our experience.

News from Cologne: Patrick Hölker

University of Cologne, Faculty of Medicine and University Hospital Cologne, Department I of Internal Medicine, Center for Integrated Oncology Aachen Bonn Cologne Duesseldorf, Cologne

Expanding high-dimensional analyses of aggressive lymphoma, the tumor microenvironment, and extracellular vesicles

In Cologne, the CRC1530 focuses on elucidating and targeting the pathogenic mechanisms that underlie B cell lymphoma, emphasizing understanding how malignant B cells and their local tumor microenvironment interact. Within this framework, our CyTOF and imaging mass cytometry (IMC) laboratory enable comprehensive analyses of tumor cells and their microenvironmental partners in mantle cell lymphoma, chronic lymphocytic leukemia (CLL), and CAR T cell-treated diffuse large B cell lymphoma. Notably, we recently supported a study revealing that LYN-kinase in leukemic cells can program stromal fibroblasts to promote leukemic survival (Vom Stein et al., Nat Commun., 2023) underscoring the critical role of high-dimensional analyses of the tumor microenvironment in B cell malignancies.

Despite these advances, numerous mechanisms of aberrant cellular communication remain poorly understood, particularly those involving extracellular vesicles (EVs). EV-mediated intercellular communication is especially challenging, as traditional bulk analyses often provide only low-dimensional insight into EV composition and their recipient-cell profiles. Consequently, the field urgently needs approaches leveraging single-cell resolution to capture the heterogeneity of EV uptake and function in life sciences research.

To address these gaps, we aim to develop novel MC- and IMC-based methodologies that afford high-dimensional single-cell analyses of EV-recipient cells, focusing on CLL. In this regard, we have developed a tellurium-based metabolic mass-tag labeling strategy that marks the proteome of secreted EVs. By applying TeLEV in various B cell disease models and validating results in patient-derived samples, we can visualize EV uptake, characterize their molecular signatures in recipient immune cells, and assess their biological impact with unprecedented resolution.

News from Berlin (DRFZ): Adrian Barreno-Sanchez

German Rheumatology Research Center Berlin – A Leibniz Institute, Berlin, Germany

A Mass Cytometry Workflow for Ex-Vivo Phosphorylation and Mucosal Immune Profiling in Systemic Lupus Erythematosusbstract

Systemic lupus erythematosus (SLE) is a prototypical systemic autoimmune disease characterized by immune tolerance breakdown and the production of IgG autoantibodies. Even though the mechanisms of disease progression, clinical manifestation and therapy response in SLE are highly heterogeneous and poorly understood, recent evidence suggests an involvement of the gut-immune axis in both SLE pathogenesis and prognosis. This highlights the need to investigate mucosa-related immunity as a potential source of patient heterogeneity.

To explore the role of mucosa-related immunity in SLE, we established a robust mass cytometry workflow for in-depth ex-vivo phosphorylation profiling and comprehensive mucosal immune phenotyping of peripheral blood. The workflow is optimized for the analysis of fixed whole blood samples, scalable to large clinical studies, and specifically designed to maximize data output from limited sample material.

The sample processing pipeline uses a tailored cell sorting strategy to divide whole blood into lymphoid and myeloid fractions, which are subsequently analyzed with two optimized >50-plex antibody panels. These panels include markers that identify key mucosal-associated immune populations, such as IgA+ plasmablasts, γδ T cells, MAIT cells, invariant NKT cells, and other leukocytes with gut-homing properties, expressing, e.g. CCR9, CD69, and the integrins α4β7 or αEβ7. Additionally, the panels incorporate phosphoprotein-specific antibodies to measure ex-vivo activity of multiple intracellular signaling pathways.

Overall, this workflows allows for deep phenotyping of peripheral blood, with focus on gut-related immune populations, and the analysis of intracellular phospho-signaling activity by mass cytometry. By providing a comprehensive view of mucosal immunity, this approach may shed light into role of the gut-immune axis in SLE and identify potential sources of disease heterogeneity that could be exploited for tailored clinical management.

News from Freiburg: Emilia Schlaak

Department of Medicine II (Gastroenterology, Hepatology, Endocrinology and Infectious Diseases), Freiburg University Medical Center, Faculty of Medicine, University of Freiburg, Freiburg, Germany

Immunotherapies using checkpoint blockade antibodies are now utilized in Hepatocellular carcinoma (HCC) patients but prediction of responding patients remains difficult. We utilize mass cytometry to profile the dynamic changes of the peripheral immune response to immunotherapy and utilize imaging mass cytometry to dissect the immune architecture in the tumor microenvironment.

I will discuss data from translational work that identifies key responding immune populations in patients exposed to checkpoint blockade in HCC. Our data indicates significant remodeling of the peripheral immune compartment involving distinct CD4 and CD8 T cell subsets. The response dynamics may help predict therapeutic outcomes and could support the development of more personalized treatment strategies for HCC.

6:00 pm – 10:00 pm Poster session & dinner

6:00 pm – 10:00 pm Poster presentations

Evaluation team: Desiree Kunkel & Sarah Warth

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Bettina Bernard

Technical University of Munich (TUM), School of Medicine, Institute for Medical Microbiology, Immunology and Hygiene, Munich 81675, Germany

In vitro induction of exhaustion in human CD8+ T cells via mitochondrial inhibition and ectopic TOX expression

In vitro manipulation of human exhausted T cells (Tex) is challenging due to the low numbers of Tex that can be isolated in human disease and absence of a consensus model to induce exhaustion. To overcome this and to recapitulate human Tex development *in vitro*, we generated a model that integrates major cues driving T cell exhaustion, such as TCR signaling, mitochondrial dysfunction induced by pharmacologic mitochondrial translation inhibition and TOX overexpression. Using CyTOF profiling, flow analysis and RNAseq analysis we identified that metabolic dysfunctionality enhances the differentiation to terminally exhausted CD127–PD-1hi cells with reduced cytokine production and gene expression programs, but also induces a significant fraction of exhausted T cell precursor populations (Tpex). Ectopic TOX expression further drives exhaustion programs with enhanced inhibitory receptor expression and reduced cytokine release. These models provide opportunities to study distinct stages of Tex development. Transcriptional analysis linked FOXO1 gene signatures to the enrichment of Tpex cells in the mitochondrial inhibition model. CRISPR knockout of FOXO1 led to a collapse of Tpex and severe Tex populations, validating the *in vitro* model as suitable to dissect the transcriptional regulators of T cell exhaustion.

2

Vera Bockhorn

Deutsches Rheuma-Forschungszentrum Berlin, a Leibniz Institute, Berlin, Germany

SplitSOM automates debarcoding of mass cytometry data

Sample barcoding offers the advantage of measuring multiple samples from different sources simultaneously, thereby effectively harmonizing assay preparation and sample acquisition conditions. Debarcoding, i.e. the extraction of single sample data from barcoded data convolutes is a crucial step in mass cytometry data curation workflows. Traditionally, debarcoding is performed by manual gating, although algorithmic solutions have also been developed for this task. However, such solutions showed suboptimal performance on our surface barcoding on live cells.

Here, we introduce SplitSOM, a novel computational debarcoding method utilizing FlowSOM, a widely accepted clustering method for cytometry data. In contrast to existing debarcoders, SplitSOM considers the entire information of cellular neighborhood based on overall barcode stainings to group cells with similar barcode signatures, rather than assessing and assigning each cell individually. Additionally, SplitSOM can automatically resolve different barcode configurations, including examples using varying numbers of barcode markers.

We evaluated the recovery and assignment accuracy of SplitSOM. In a 20-sample dataset with a 6-choose-3 barcode configuration, the events-to-sample assignment rate was 92%, closely matching the results of manual gating. We further investigated the rate of cell misclassification after successful SplitSOM debarcoding by analyzing a secondary (CD45) barcode not used for debarcoding. We assessed the cross-contamination between three samples, each stained with a different CD45 barcode. The cross-contamination was less than 0.17%, indicating minimal contamination between the debarcoded samples.

In summary, our newly developed SplitSOM debarcoder permits quick, flexible, accurate and efficient debarcoding of mass cytometry data, facilitating swift and automated data curation in high throughput facilities.

3

Martin Borgmann

Department of Internal Medicine II, Universitätsklinik Freiburg, Universität Freiburg, 79106 Freiburg, Germany

Imaging Mass Cytometry Protocol for Formalin-Fixed Paraffin-Embedded Mouse Tissues: Challenges and Opportunities in Analyzing Archival Specimens

Imaging mass cytometry (IMC) has recently become a widespread method for spatial analysis of immune and stromal components in a range of diseases, including solid tumors. The majority of studies have focused on patient samples. In contrast, the application of IMC to animal tissues remains limited, including the gastrointestinal tract of C57BL/6 mice.

Here we established a highly multiplexed IMC panel to apply IMC on formalin-fixed paraffin-embedded (FFPE) gastrointestinal mouse tissues, with the goal to specifically interrogate stomachs of the B6.IL-1beta(EBV)tcw mouse model, which is used to study gastroesophageal adenocarcinoma development. To address challenges associated with older FFPE-blocks, our approach encompasses specimen collection, fixation, antibody validation via immunofluorescence, metal tag conjugation, and IMC validation, as well as validation for IMC analysis. The intricacies of working with FFPE tissues include insights into specimen processing, antigen retrieval, and blocking steps to enhance data quality. We tested over 70 antibodies using the Hyperion® imaging system with a 63% success rate of obtaining IMC signal after in-house conjugation. Our designed signature panel allows for spatial analysis of distinct cell types (mesenchymal, stromal, immune, epithelial), and processes involved in cancer development including epithelial-to-mesenchymal transition, a wide range of immune cell processes (e.g. polarization and cytotoxicity), signaling pathways, as well as the overall tissue architecture (E-Cadherin, Fibronectin, Collagen and β-tubulin 3).

Our work will serve as a resource to explore immunological and stromal landscapes of existing murine FFPE specimens using highly multiplexed imaging. This protocol allows for analyzing older FFPE blocks with IMC technology, leveraging these resources to accelerate scientific discovery.

4

Sebastian Ferrara

Deutsches Rheuma-Forschungszentrum Berlin, a Leibniz Institute, Berlin, Germany

NFDI4Immuno: Building a framework for comprehensive immunological data integration and analysis, collaboration and Open Science

In the era of advanced single-cell technologies, particularly mass cytometry, capable of generating very information-rich datasets, the necessity for data sharing and reuse has grown significantly in immunological research. To address this need, the National Research Data Infrastructure for Immunology (NFDI4Immuno) initiative has been founded.

Here, we present how the NFDI4Immuno initiative aims to integrate immunological data and metadata from various experimental technologies, including cytometry, sequencing, immunoassays, and imaging, to provide a holistic view of immunological processes. The initiative strives to enhance scientific collaboration by harmonizing data representations, metadata standards, ontologies, and programmatic interfaces with other NFDI consortia, promoting seamless queries and cross-referencing. The project is committed to support users in effectively utilizing its resources and to foster the adoption of FAIR principles (findability, accessibility, interoperability and reusability) within the immunological community to contribute to the broader cultural shift towards Open Science. Finally, NFDI4Immuno plans to establish and manage a network of federated repositories for immunological data, to develop tools and services that facilitate standardized and reproducible data analyses, reinforcing scientific rigor and transparency.

5

Leo Fiebig

Deutsches Rheuma-Forschungszentrum Berlin, a Leibniz Institute, Berlin, Germany

Antibody Isotype and Subclass Expression Shapes Plasma Cell Heterogeneity in the Human Bone Marrow

Mature plasma cells in the human bone marrow (BMPCs) are essential for humoral immunity and memory and may contribute to autoimmunity through the secretion of autoantibodies. Significant phenotypic heterogeneity among BMPCs has been reported, including the loss of traditional B cell markers like CD19 and the expression of atypical receptors such as CCR2, CD56, and CD28. However, the drivers and implications of this heterogeneity remain poorly understood.

We conducted an in-depth characterization of human bone marrow plasma cells (BMPC) of 9 donors using a 48-marker mass cytometry assay resolving all human Ig isotypes and subclasses, and targeted single-cell sequencing from 4 donors, including a 62-marker AbSeq panel, quantification of 399 mRNAs, and BCR sequence information, together enabling detailed investigation of BMPC heterogeneity in relation to Ig isotype expression. IgG (48%) was the most abundant isotype among BMPCs, followed by IgA (41.5%) and IgM (3.2%). Within the IgG+ BMPC population, IgG1 and IgG2 were predominant, while IgG3 and IgG4 were detected at lower frequencies. Notably, IgG1+ and IgM+ BMPCs were enriched in CD19neg BMPC, a phenotype previously linked to PC longevity. Additionally, IgG1+ BMPCs were enriched in a novel CD19-CD56+ subset, whereas IgA1+ BMPCs were more common among CD19+CD45- BMPCs. Ongoing analyses aim to systematically map the relationship between BMPC phenotype and Ig subclass, linking these characteristics to IgVH properties to elucidate clonal relationships and levels of somatic hypermutation across BMPC phenotypes. Both assays provided consistent information, underlining the validity of the results obtained by mass and genomic cytometry.

Our findings reveal that BMPC phenotypes are strongly associated with Ig isotype and subclass expression. This suggests that BMPC phenotypes and potentially their functions are pre-determined during class-switch recombination (CSR) in germinal centers Thus, CSR may not only have a critical role in defining antibody function but also in shaping the phenotypes of BMPC, which may regulate PC localization and persistence of individual PC in the bone marrow.

6

Yola Gerking

Department of Clinical Medicine, University of Bergen, Bergen, Norway and Department of Neurology, Neuro-SysMed, Haukeland University Hospital, Bergen, Norway

CNS Immune Profiling and Microglial Dynamics in Progressive MS Using Mass Cytometry

Multiple Sclerosis (MS) is a chronic inflammatory condition of the central nervous system (CNS) characterized by myelin loss and axonal damage. Most patients initially experience relapsing-remitting MS (RRMS), involving episodes of immune cell infiltration and inflammation in demyelinating lesions, followed by remission. Progressive forms, including primary progressive MS (PPMS) and secondary progressive MS (SPMS), lead to gradual disability without relapses, driven by CNS-restricted inflammation. Understanding the role of CNS-resident microglial cells in these processes is critical in progressive MS.

This study employs imaging mass cytometry (IMC) to analyze immune cells in cerebrospinal fluid (CSF) and post-mortem CNS tissues from progressive MS patients. An extensive microglial phenotyping panel, including TMEM119, MerTK, P2Y6, Iba1, and CD68, was developed to profile microglial activation states in white matter smouldering lesions and iron rims. Validation included microglial cell lines, iPSC-derived microglia, PBMCs, tonsils, healthy brain tissue, and MS-affected brain tissue.

Preliminary results show the panel distinguishes resting microglia, activated microglia, and infiltrating macrophages. Activated microglia exhibit a pro-inflammatory phenotype, enhancing understanding of CNS inflammation in progressive MS and RRMS patients with smouldering lesions.

7
Julia Hecker
Charité - Universitätsmedizin Berlin
no publishing

8
Justin Jakull
Jena University Hospital, Friedrich-Schiller-University Jena, Jena, Germany

How to use iodinated Hoechst reagent in suspension mass cytometry?

Cell identification in mass cytometry relies on their sufficient labeling with a metal-labeled probe, which is routinely achieved by post-permeabilization staining with an iridium-labeled DNA intercalator. The detection of doublets is usually achieved by using the DNA content and event length but this practice is not sufficient. Cell membrane permeant Hoechst dyes are commonly used in flow cytometry to detect selectively live nucleated cells. By modifying Hoechst dyes with a metal isotope it can be used for mass cytometry approaches as well.

Here we analyzed whole blood samples stained with commercially available Maxpar DIPA-kit including 127I-Hoechst dye only as well as in combination with iridium intercalator in order to compare the performance to samples stained exclusively with iridium intercalator.

Preliminary data show a rather limited discrimination of doublet cells when incubating 127I-Hoechst with antibodies. However, this shortened protocol allows to circumvent the permeabilization step in sample preparation. Results show similar frequencies to iridium-stained samples for all major leukocyte subsets. When 127I-Hoechst is utilized in addition to iridium intercalator the differentiation of doublets from single cells is more effective. As a result we suggest 127I-Hoechst only staining for applications requiring same-day analysis such as antibody titrations and panel testing. For use cases such as clinical studies in which a better resolution between doublets and singlets is desired, an adoption of 127I-Hoechst staining into standard iridium-based protocol is recommended. Altogether, we propose to include 127I-Hoechst into mass cytometry assays.

9
Rafael Käser
Department of Internal Medicine II, University Hospital Freiburg, Germany

CD161 expressing CD4+CXCR3+ T cells accumulate in the liver of obese patients with MASH

Background: Metabolic dysfunction-associated steatotic liver disease (MASLD) is emerging as a leading cause of chronic liver disease in western countries. Hepatic steatosis can progress to inflammation (Metabolic Dysfunction-associated Steatohepatitis or MASH), with a suspected role of hepatic T cells driving this process.

Methods: The study involved 51 obese patients undergoing bariatric surgery, stratified into groups with few (n=33) and high (n=18) inflammation levels according to histologic scoring and clinical parameters. Liver and blood samples were characterized by flow cytometry, validated by mass cytometry and subsequently further investigated by single cell RNA sequencing for a broad range of immune cell subsets.

Results: CD4 T cells were significantly more abundant in inflamed liver tissue. Cluster analysis identified a distinct CD161-expressing CD4+CXCR3+ T cell subset enriched in high-inflammation patients. In contrast, CD8 T cells and other immune cells showed no significant differences between the groups. Mass cytometry revealed additional defining markers: CD26, PD-1, CD127, and Tbet. Functional analysis showed increased pro-inflammatory cytokines and cytotoxic granules (Granzyme B and IL-17A) in liver and blood. These inflammatory changes were dynamic, subsiding over 12 months post-weight loss. Furthermore, significant positive correlations could be observed between the specific inflammatory T cell subsets and clinical and histological parameters indicative of liver damage.

Conclusions: Our findings reveal critical immunological mechanisms in obesity-related liver inflammation. The CD161-expressing CD4+CXCR3+ T cell population emerges as a potential diagnostic and therapeutic target for MASLD. The inflammatory changes' reduction after weight loss suggests targeted interventions could modulate disease progression, offering promising insights into modulating metabolic liver disease.

Melissa Klug

Standard BioTools

10

Whole Slide Imaging Modes and Curated Antibody Panels for Imaging Mass Cytometry Approach Reveal Extensive Spatial Heterogeneity of Human Glioblastomas

Glioblastoma (GBM) presents a complex form of brain cancer that is challenging to diagnose and treat. Gaining spatial insights into the cellular composition of GBM tissue has tremendous potential to inform clinicians and researchers about mechanisms behind spatial predictors of treatment success and disease etiology and progression.

Imaging Mass Cytometry™ (IMC™) is a high-plex spatial biology imaging technique that enables deep characterization of the diversity and complexity of GBM and other tumor microenvironments (TMEs). IMC supports detailed assessment of cell phenotype and function using 40-plus metal-tagged antibodies simultaneously on a single slide without artifacts associated with fluorescence-based spectral overlap, tissue autofluorescence, multiple acquisition cycles and tissue degradation.

Specifically designed for high-throughput applications and whole slide imaging (WSI) modes, the Hyperion XTi™ Imaging System with 40-slide loader permits automated and continuous imaging of more than 40 large tissue samples (400 mm2 per tissue) per week. We showcase the application of WSI using curated antibody panels to study the complexity of the GBM TME.

Nisarg Dobaria

Department of Dermatology, University Hospital Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany

11

Cross talk between skin and joints impairs bone homeostasis in psoriatic arthritis

Psoriasis and psoriatic arthritis (PsA) are chronic inflammatory disorders with interconnected pathologies involving the skin and joints. Innate lymphoid cells (ILCs) have emerged as key players in the transition from skin inflammation to joint destruction, yet the mechanisms underlying this „skin-joint axis“ remain incompletely understood. This study leverages Imaging Mass Cytometry (IMC) to investigate the spatial distribution and chemokine receptor expression of ILCs in lesional and non-lesional skin from psoriasis patients with and without PsA.

Our previous flow cytometry analysis of peripheral blood mononuclear cells (PBMCs) from psoriasis patients and healthy controls revealed a significant increase in ILCs in patients, with elevated expression of the chemokine receptor CXCR3 in ILC2s, a receptor linked to immune cell recruitment to inflamed tissues. Extending these findings to the psoriasis tissue microenvironment using IMC, we observed high CXCR3 expression in ILCs identified within the lesional skin. Additionally, CXCL16, a skin-homing chemokine, was predominantly expressed in the epidermis and in monocytes. These observations suggest key roles for CXCR3 and CXCL16 in mediating ILC migration and tissue infiltration. Future comparative analyses with PsA lesional tissue will focus on these chemokines to better understand the migratory nature of ILCs from skin to joints.

This spatially resolved approach provides comprehensive immune profiling of psoriatic skin, comparing lesional and non-lesional sites with healthy controls to identify early disease-driving factors. By linking IMC data to clinical PsA status, we aim to uncover biomarkers predictive of disease progression and elucidate mechanisms facilitating the migration of skin-derived immune cells to joint tissues.

12

Günter Päth

Translational Systems Immunology, Hepatogastroenterology, Clininc for Internal Medicine II, University Hospital Freiburg

First impression of the new transparent polymer-coated slides for use in multiplex ion beam imaging (MIBI) mass cytometry

Introduction: The MIBIscope is a recent addition to the field of tissue imaging by mass spectrometry using time-of-flight secondary ion beam imaging for simultaneous detection of 40+ metal-labeled antibodies at subcellular resolution down to 0.39 µm per pixel. The MIBIscope works with a 30keV-activated primary xenon ion beam, which is grounded by a conductive gold coating on the slide. However, the opaque gold layer does not allow traditional light-based approaches in addition to standard MIBIscope imaging. To overcome this issue, slides with a conductive but transparent blue top layer have been developed. We have tested the performance of two pre-production blue slides and compared them with the gold standard.

Methods: Four µm thick sections of a tissue microarray (TMA) were cut, mounted and stained in parallel using a single antibody mastermix. After individual background removal, blue and gold data were segmented and analysed in parallel.

Results: Blue slides showed a trend for better tissue adhesion. Blue slides showed slightly less background noise and slightly higher signal intensity before and after background removal. Consequently, segmentation and analysis of blue slide data resulted in a slightly lower number of undefined cells in unsupervised clustering and subsequently better segregation between immune cells and non-immune cells.

Conclusion: Compared to gold slides, blue slides offer transparency and, at first impression, better tissue adhesion, slightly less background noise and slightly improved signal intensity. Segmentation and clustering are improved accordingly.

13

Pr Tang

Division/Center of Pediatric Hematology/Oncology at Children's Hospital of Zhejiang University School of Medicine, China

Mass Cytometry in Pediatric Hematological Research and Potential Clinical Application

Substantial progress of high content cytometric detection has been made in pediatric hematology-oncology diagnosis in recent years. Defining complex antigenic profiles that are associated with specific cytogenetic/molecular defects, as well as systemic immunomonitoring for programmed chemo-immuno treatment, has significantly contributed to more accurate prognosis and improved efficacy. Mass cytometry holds a promise for being a more standardized and comprehensive cytometric detection technology. In this talk, we would like to discuss it's current and future applications in hematological research and diagnosis, combined with novel bioinformatic toolbox and immunotherapeutic interventions.

Qaisar Akram

Department of Pathobiology (Microbiology), University of Veterinary and Animal Sciences, Lahore (Narowal Campus) Narowal 51600, Pakistan

14

Silver Nanoparticles Coated AZ31: A Nanotech Defender in the War Against Foot-and-Mouth Disease Virus

Background: The economically important, foot-and-mouth disease (FMD) is extremely contagious disease of cloven-hoofed animals caused foot-and-mouth disease virus (FMDV). There isn't an antiviral treatment available for FMDV right now. The bactericidal effect of Ag NPs/PEI/MAO (APM) coated AZ31 is well established. The purpose of this work is to evaluate the APM coated AZ31's *in vitro* antiviral activity against FMDV.

Methodology: Using the Baby Hamster Kidney (BHK-21) cell line cultured in GMEM medium supplemented with 10% Fetal Bovine Serum, the cytotoxicity and antiviral characteristics of APM coated AZ31 will be observed. APM coated AZ31 will be prepared according to Xin Wang et al., 2021. Using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide test, the viability of the cells will be assessed. The culture medium was removed, and ten-fold serial dilutions of FMDV in a serum-free medium were added and then incubated for one hour at 37°C. The plaque reduction assay will be used to assess the direct effects of the FMDV strain O/ME-SA/PanAsia-2/ANT-10 on APM coated AZ31 in both the extracellular (virucidal assay) and different stages of virus replication (antiviral assay) phases. The Nano-Particles (NPs) will be introduced to the cells at the pre-attachment, attachment, and post-penetration stages of the viral infection cycle to ascertain the mode of their antiviral activity. The NPs will be dissolved in distilled water and utilized at doses of 25, 50, 100, 150, 200, and 250 µg/ml to evaluate the antiviral activity.

Results: Anticipated outcomes indicate that APM-coated AZ31 will be safe in BHK-21 cell line at concentrations up to 250 µg/ml. The NP treatments suggested that the APM coated AZ31 would have antiviral and virucidal effects *in vitro*. APM coated AZ31 can inhibit FMDV by more than 90% during the early phases of infection, such as attachment and penetration, but not after penetration, according to the plaque reduction assay.

Conclusion: The inference made is that APM coated AZ31 may be administered locally as an antiviral medication to susceptible animals in the early stages of infection.

Marius Schwabenland

Institute of Neuropathology, Faculty of Medicine, University of Freiburg, Freiburg, Germany

15

High throughput spatial immune mapping reveals an innate immune scar in post-COVID-19 brains

The underlying pathogenesis of neurological sequelae in post COVID-19 patients remains unclear. Here, we used multidimensional spatial immune phenotyping methods on brains from initial COVID-19 survivors to identify the biological correlate associated with previous SARS-CoV-2 challenge. Compared to healthy controls, individuals with post COVID-19 revealed a high percentage of TMEM119+P2RY12+CD68+Iba1+HLA-DR+CD11c+SCAMP2+ microglia assembled in prototypical cellular nodules. In contrast to acute SARS-CoV-2 cases, the frequency of CD8+ parenchymal T cells was reduced, suggesting an immune shift towards innate immune activation that may contribute to neurological alterations in post COVID-19 patients.

16

Niklas Vesper

Department of Medicine II, University Hospital Freiburg – Faculty of Medicine, University of Freiburg, Freiburg, Germany and Faculty for Biology, University of Freiburg, Freiburg, Germany

ISO: Image analysis of Subcellular localization and spatial Overlap in tissue cells

Generalist segmentation algorithms like Cellpose [Pachitariu, M., & Stringer, C. et al. (2022)] and DeepCell Mesmer [Greenwald, N. F. et al. (2022)] enabled fast and reliable tissue segmentation. Yet these segmentation algorithms do not fully cover analysis of subcellular localization of proteins, since they only take into account segmentation masks of the full cell and the nucleus. Here, we developed an algorithm for the detailed analysis of subcellular localization based on image coordinate information and a python based analysis pipeline to accommodate this algorithm.

As application examples, we analyzed the nuclear translocation of the cAMP dependent transcription factor 6 (ATF6) and nuclear versus cytoplasmic localization of the focal adhesion kinase (FAK) in hepatocellular carcinoma. Where we could show improved characterization.

Another benefit of this approach was the increased sensitivity in transcription factor analysis of immune cells, e.g., FoxP3 in Tregs. By this a more comprehensive and detailed profiling of the cellular landscape and a better understanding of the cellular state of immune cells in the tissue was achieved. Hence, the ISO algorithm provides a versatile tool for imaging analysis not only enabling determining subcellular localization of proteins but also to increase the detection sensitivity.

Survey for the 8th GMCUF

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Wednesday, 12th of February 2025

9:00 am – 9:30 am Product feature talk Standard BioTools

Chairs: Sabine Baumgart & Claudia Peitzsch

Benjamin Ehret
Data Scientist, Navignostics AG

Enhancing IMC through AI-based information transfer between antibody panels

The emergence of multiplexed protein imaging technologies, such as imaging mass cytometry, has enabled the analysis of tumor tissues at an unprescedented level of detail. However, the number of proteins of interest still exceeds the number of available imaging channels. One way to increase the number of analyzed proteins is to image consecutive tissue slices with different antibody panels. Yet, this approach has the limitation that one cannot analyze the spatial interactions of cells stained with the different panels. To address this issue, we use deep learning to transfer information between the two multiplexed images. In this talk, I will present an application of this approach to a colorectal cancer data set, imaged with a tumor panel and an immune panel. We use the two panels to define different sets of cell types and show that we can train a neural network to infer immune cell types in the tumor panel image and vice versa. This information transfer allows us to perform spatial analysis of detailed immune and tumor cell types, leading to a more comprehensive understanding of the analyzed tissues.

9:30 am – 10:45 am Imaging MC and Data Session

Chairs: Bertram Bengsch & Desiree Kunkel

Invited Talk by Carl Lee, Oxford, UK
University of Oxford, UK

Advancing Spatial Biology: Unlocking Clinical Potential with Spatial Proteomics

A 2025 Nature Biotechnology editorial spotlighted the rapid advancements in spatial biology technologies, emphasizing the superior clinical potential of spatial proteomics over transcriptomics. Spatial transcriptomics struggles with scalability and limited gene coverage, whereas spatial proteomics stands out for its scalability and capacity to deliver direct functional insights. A significant breakthrough emerged from combining mass cytometry with imaging (IMC), enabling multiplexed protein detection at unprecedented resolution. Leading companies like IonPath and Standard BioTools are advancing this field through automated high-throughput IMC platforms.

Despite challenges such as high instrument costs and the need for standardization, our work at Oxford demonstrates the transformative potential of spatial proteomics in understanding myocardial infarction. By mapping ischemic and border zones at single-cell resolution in treatment versus non-treatment models, this approach has revealed distinct spatial signatures of cardiac protection versus adverse remodeling, offering new therapeutic insights. Collaborating with industry partners, we have developed a roadmap for utilizing IMC technology for potential drug validation. The integration of multimodal approaches, combining spatial transcriptomics and proteomics, suggests that spatial biology will become the next standard, particularly in cardiovascular and oncology applications.

Short Talk by Huck Adrian, Berlin, Germany,

Charité – Universitätsmedizin Berlin, Department for Gastroenterology, Infectious Diseases and Rheumatology, Germany

Fatty acid metabolism-dependent polarization of tumor associated macrophages

Colorectal cancer (CRC) is one of the most common cancers and the second leading cause of cancer-related deaths worldwide. An interplay between tumor cells and surrounding stromal and immune cells characterizes solid tumors such as CRC. One prominent immune cell type is tumor-associated macrophages (TAMs), which typically display a pro-tumorigenic and anti-inflammatory phenotype. Previous studies have shown a negative correlation between overall survival and the abundance of specific TAM subsets in CRC. Our group recently described a mechanistic link between the uptake and metabolism of fatty acids and the polarization of macrophages towards a pro-tumorigenic and immunosuppressive phenotype. To study the presence and metabolism of TAMs, we established a 36-plex imaging mass cytometry (IMC) panel covering immune cell, cancer-specific and metabolic markers on formalin-fixed and paraffin-embedded (FFPE) samples. Using this panel, we analyzed a cohort of CRC patients, including samples of healthy areas, tumor center and infiltrating margin, organized in tissue microarrays. Additionally, we applied a 7-plex immunofluorescence panel on consecutive tissue sections to study the presence of lipid droplets using ADRP, a structural protein of lipid droplets, as well as epithelial and major immune cell markers. By combining both methods, we were able to identify lipid droplet-bearing cells and correlate their presence to the deep immune phenotyping and the activation of metabolic pathways in various immune cell subsets. Subsequently, these cellular and metabolic features were correlated with clinical parameters such as prognosis. The understanding of fatty acid metabolism of tumor-infiltrating immune cells creates new possibilities for cancer-targeted therapeutic interventions.

Short Talk by Ira Godbole, Freiburg, Germany

Charité – Universitätsmedizin Berlin, Department for Gastroenterology, Infectious Diseases and Rheumatology, Germany

Deep profiling of the tumor immune microenvironment for personalized treatment for biliary tract cancer

Background and Aims: Biliary tract cancers (BTCs) represent a heterogeneous group of rare, aggressive malignancies with low survival rates and increasing incidence. 3 BTC patients that progressed under first-line chemotherapy were screened by next generation sequencing for tumor mutational profiling and determined to have homologous recombination deficiency due to mutations in BRCA and BAP1 and were thus treated with PARP inhibitors. This study sought to understand if analysis of the tumor immune microenvironment (TiME), may provide additional insights into the suitability of immunotherapy options. We thus performed IMC analysis before PARP treatment and determined the tumor-immune architecture.

Methods: Tumor samples from three BTC patients subsequently treated with a PARP inhibitor targeting specific DNA damage pathway mutations and exhibiting distinct clinical outcomes, were selected for highly multiplexed imaging mass cytometry (IMC) (42 markers, 3 ROIs each from tumor, margin and adjacent regions of BTC resections).

Results: Clinically, two out of three patients with BRCA mutations showed complete remission to PARP inhibitors, in contrast, the patient with BAP1 syndrome experienced significant tumor progression. IMC analysis revealed distinct marker expression patterns in tumor, margin, and healthy tissues, including CA19.9, TROP-2, and Claudin18.2 in tumor regions, with HePar expression absent. Immune cells were present in both tumor and stromal regions, with notable differences in immune cell populations. The BAP1-mutated sample exhibited high immune cell infiltration, including activated CD8 T cells (TRM and TEX subsets), along with Granulocytes, NK cells, CD4 memory T cells and B cells. In contrast, immunosuppressive populations such as M2-like tumor-associated macrophages, Tregs, and MDSCs were enriched in BRCA-mutated samples. We applied a spatial immune classification developed for primary liver cancer, indicating an enriched immunotype in the BAP1-mutated sample and a depleted immunotype in the BRCA-mutated sample. Subsequent treatment with anti-PD-1 and TKI therapy for the BAP1-mutation patient resulted in a partial response and longterm survival.

Conclusions: The tumor immune architecture appears to be connected to different genetic drivers of homologous mismatch repair insufficiency in BTC. High infiltration with immune cells in the BAP1 patient was observed in the context of response to anti-PD-1 / TKI therapy. Spatial profiling of the tumor-immune architecture may synergize with mutational profiling for improved personalized decision making in BTC patients.

Short talk by Matthieu van Tilbeurgh, Fontenay-aux-Roses, France

Charité – Universitätsmedizin Berlin, Department for Gastroenterology, Infectious Diseases and Rheumatology, Germany

Role of antigen bio-distribution and persistence in early and long-term immune responses to the yellow fever vaccine in non-human primates

Purpose: Despite recent advances in vaccine development, gaps remains to understand the mechanisms driving long-term memory response to vaccines. The purpose of the work is to characterize the role of vaccine antigen persistence in generating durable immunity. Using the live-attenuated yellow fever (YF)-17D vaccine, one of the most efficient and durable vaccine, we explored molecular and cellular interactions in tissues of immunized non-human primates.

Methods: Two groups of cynomolgus macaques were either vaccinated with a commercial dose of live-attenuated vaccine or with the same dose of a β-propiolactone-inactivated vaccine adjuvanted with alum. Animals also received a 5-Iodo-2'-deoxyuridine (IdU) treatment to follow the mobilization and persistence of immune cells following immunization. Longitudinal systemic immune responses were characterized by mass cytometry, as well as viral load by qPCR, and neutralizing antibodies. At necropsy, different types of tissues were collected at several timepoints to follow antigen persistence by immunohistofluorescence (IHF) and in situ hybridisation; and to explore tissue immune responses by imaging mass cytometry (IMC). Results were compared to data obtained from tissues of macaques infected wild type Asibi strain.

Results: Low and transient viremia is observed in vaccinated animals (reaching 103 to 104 copies/mL) at approximately day 3 pi. Longitudinal analysis of immune responses by mass cytometry shows a high mobilization of innate and adaptive immune cells at early time-points. Adaptive T and B cells show persistent activation in the long term (up to 12 month pi) as demonstrated by the persistence of IdU+ cells. Neutralizing antibodies were shown to persist for the live-attenuated vaccine whereas they decreased significantly around 3 months pi for the inactivated vaccine. By comparing these strategies, we identified differences in regulatory T cells and three types of circulating follicular helper cells (CD127+ PD-1-, CD127mid PD-1- CD25+, and CD127lo PD-1+) that could affect antibody persistence. In the tissues, YF antigens were detected at early timepoints mainly in the liver, although at lower rates than the infected animals, and in the spleen.

Conclusion: Our data enhance the understanding of the immunological mechanisms of YF-17D vaccination. Together, they highlight the relevance of specific immunological mechanisms that occur after vaccination.

10:45 am – 11:30 am Coffee break

11:30 am – 1:00 pm News from … Part 2

Chairs: Sabine Baumgart & Lena Müller

News from Wien: Klára Brožová

Medical University of Vienna, Vienna, Austria

Spatial proteomics to reveal intratumoral heterogeneity in breast cancer subtypes induced by the tumor microenvironment

Breast cancer (BC) remains a global health challenge, impacting a significant portion of the female population. Understanding proteomic heterogeneity within BC is crucial for improving diagnostic accuracy and therapeutic efficacy. Imaging Mass Cytometry (IMC) of tumor xenograft models provides high spatial resolution and targeted protein analysis within BC tissue architecture but is limited in multiplexing. In contrast, Imaging Mass Spectrometry (IMS) enables broader molecular profiling with higher multiplexing capabilities, but limited in spatial resolution and confidence of identification.

Human BC cell lines (MCF-7, SKBR-3, MDA-MB-231) were inoculated into female athymic BALB/c-nude mice. Excised tumors were embedded in gelatin or paraffin, or were snap-frozen in liquid nitrogen. One tissue was allocated for IMC and an adjacent one for IMS. An optimized IMC panel, selected based on literature review and a bulk proteomics experiment, revealed clear heterogeneity between BC subtypes and within tissues, demonstrating inter- and intra-tumoral heterogeneity. IMS explored the broader proteomic landscape and identified more candidate proteins. Bulk tissue LC-MS/MS identified approximately 17,000 proteins, enabling validation of IMS signals.

Integration of IMC and IMS datasets allowed spatial and molecular correlation. Segmentation maps aligned with histological assessments and distinguished tumor, stroma, and necrotic regions. Unsupervised U-MAP clustering revealed distinct molecular regions with unique proteomic profiles.

Combining IMC and IMS provided a comprehensive characterization of BC heterogeneity, identifying spatial proteomic variation not detectable by conventional methods. As next steps we aim to combine these translational research results with *in vivo* PET/MRI data to develop non-invasive diagnostic tools and enhance personalized therapeutic strategies.

News from Dresden: Ezgi Senoglu

Center for Regenerative Therapies (CRTD), TU Dresden, Germany

Epi-CyTOF-based investigation of the epigenetic state of the human developing neocortex

The neocortex is the brain structure attributed to higher cognitive functions in humans. Its development is governed by spatiotemporal gene expression programs regulated by epigenetic mechanisms, such as post-translational modifications of histones. Specific histone modifications act on genomic loci to repress or promote gene expression, thereby contributing to the tuning of proliferation and differentiation of neural progenitor cells. Given the large number of epigenetic modifications, we currently lack an understanding of the epigenetic state of neural cell populations beyond a few well-studied histone modifications. Moreover, the interplay of these modifications and their temporal changes in the developing human neocortex remain largely uncharacterised. To unravel the complexity of the epigenome during neurogenesis at single-cell resolution, we employed cytometry by time of flight with a comprehensive epigenetic panel spanning 30 different epigenetic markers, referred to as Epi-CyTOF. In human primary tissue and human cortical organoids, we were able to dissect different cell populations. Moreover, our data reveals distinct epigenetic marking in neural progenitor cells and neurons in both tissues. Among the most differentially enriched epigenetic marks, we find H3K27me3, a repressive epigenetic modification deposited by the Polycomb repressive complex 2 (PRC2), to be strongly enriched in postmitotic neurons, whereas the activating epigenetic mark H3K4me2/3 is less abundant in intermediate progenitor cells compared to other cell types. Altogether, Epi-CyTOF presents a powerful technology to decipher the complexity and dynamics of histone modifications during brain development. In the future, it will be applied to elucidate epigenetic changes in human neurodevelopmental disorders caused by mutations in epigenetic modifiers.

News from Heidelberg: Felix Hartmann

German Cancer Research Center (DKFZ), Heidelberg, Germany

Spatial quantification of cellular metabolism identifies metabolic niches predictive of response to immune checkpoint inhibition in metastatic melanoma patients

Metastatic melanoma remains challenging to treat despite advances in immune checkpoint inhibition (ICI). Clinically-relevant immune features such as T cell exhaustion and immunosuppressive polarization of myeloid cells are influenced by the cellular metabolic state. However, the metabolic landscape of the human tumor microenvironment and its implication on ICI response remain poorly understood. Here, we integrate single-cell metabolic regulome profiling (scMEP) with multiplexed ion beam imaging (MIBI) to dissect the spatial metabolic heterogeneity in the tumor microenvironment of ICI-naïve metastatic melanoma patients. At the single-cell level, we found that $CD8^+$ T cells in responders display unique metabolic states marked by balanced mitochondrial and glycolytic activity, contrasting with terminally exhausted states prevalent in non-responders. Leveraging the spatial context, we identified conserved metabolic niches in human melanoma that transcend lineage boundaries and stratify patients into distinct response groups. In addition, presence of these metabolic niches was associated with immunosuppressive myeloid cell polarization. Together, our findings reveal a novel axis of therapeutic vulnerability by linking spatial metabolic organization to ICI response. These insights pave the way for metabolic interventions that could synergize with existing immunotherapies, potentially transforming treatment paradigms for metastatic melanoma.

News from Berlin (MPIMG): Anika Rettig

Max-Planck-Institut für Molekulare Genetik, Berlin, Germany

Quantifying IMC data analysis step by step: a comparative evaluation of MICCRA and traditionally used approaches

Imaging Mass Cytometry (IMC) enables the spatial co-detection of 40+ proteins, which makes it a technology that has high application potential, enabling deep insights into tissue architecture and disease states. Simultaneously, IMC data is often very challenging to analyse. In recent years, several end-to-end data analysis pipelines have been published: SIMPLI, MCMICRO, imcRtools in conjunction with steinbock, and SPEX. Those pipelines are made for batch-processing of multiplexed images in general, and come at different levels of interactiveness. We introduce MICCRA (Modular IMC Cell Characterization with automatic Region Assembly), a modular end-to-end pipeline which in contrast to those pipelines focuses only on the analysis of IMC data, allowing a higher degree of automation. MICCRA also introduces novel contributions to IMC data analysis: automated stitching and normalisation of regions of interest (ROIs), the implementation of a distance-based denoising method by Keren et al., and performance-improving adaptations to the commonly used clustering package FlowSOM.

Our work focuses on the quantitative evaluation of the results of the steps of IMC data analysis, which is a challenging and often overlooked aspect of IMC related research. We compare MICCRA to other approaches, create groundtruth data or devise approximative quantification metrics. We show that Mesmer and the IMC Segmentation pipeline produce the best segmentation results compared to three other tools, while the output of the IMC Segmentation pipeline corresponds better to biological expectations and exhibits no reduced performance when dense tissue is segmented. Using groundtruth data of spatial T cell location, we observe the surprising result that even with the best segmentation approach, only 85% of T cells can correctly be phenotyped in our example superROI. Finally, we present adaptations to traditionally used clustering strategies. Firstly, we employ a flexible cofactor arcsinh data transformation, which succeeds in integrating clustering features from markers of all different intensity scales. Secondly, we present evidence of reduced reproducibility of clustering results based on a single SOM fitting, and that calculating a consensus result of more than 500 SOMs stabilises the result.

News from Erlangen: Aleix Rius Rigau

Friedrich-Alexander University Erlangen-Nuremberg, Erlangen, Bayern, Germany

Imaging mass cytometry-based characterisation of fibroblast subsets and their cellular niches in systemic sclerosis

Systemic sclerosis (SSc) is an autoimmune fibrotic diesease affecting the skin and internal organs. Fibroblasts are recognized as the key effector cells in the fibrosis. Over the last decade, have been shown that fibroblasts are a heterogeneous cell population with phenotypical and functional differences. However, a complete comprehensive picture is missing. Moreover, only approaches requiring tissue disaggregation have been used, losing the spatial information.

WeusedImagingMassCytometryasaspatialproteomic technique to characterize fibroblasts subsets in skin biopsies of SSc patients and healthy controls. We have identified 13 distinct fibroblast subsets, of which five were increased in SSc (Myofibroblasts, S1PR+, FAPhigh, THY1+ADAM12highPU.1high and ADAM12+GLI1+) and three were decreased (THY1+ADAM12low, TFAMhigh and PI16+FAP+). The spatial localization within the dermis differs between subsets: TFAMhigh and ADAM12+GLI1+ fibroblasts are mainly located in the upper dermis, being the first increased in healthy donors and the second in SSc patients; myofibroblasts and FAPhigh are exclusively found in the lower dermis; S1PR+ and THY1+ADAM12highPU.1high are increased in both dermal layers in SSc. Subsequently, an interaction analysis was conducted. The subepithelial space consists of TFAMhigh fibroblasts in healthy and ADAM12+GLI1+ fibroblasts in SSc. Furthermore, the S1PR+ fibroblasts exhibits a complete shift in their neighbourhood network in SSc compared to healthy. Lastly, the percentage of S1PR+ and their neighbouring ADAM12+GLI1+ fibroblasts are significantly associated with clinical outcomes of SSc. This indicates that not only are the cell subsets of significance in the disease development, but also their spatial localization and relationships.

News from Freiburg II: Florian Ingelfinger

Weizmann Institute of Science, Rehovot, Israel and Medical Center-University of Freiburg, Freiburg, Germany

CytoVI: Deep generative modeling of cytometry data across technologies

Flow cytometry was historically the first single cell technology to measure millions of cellular states within minutes. Due to its robustness and scalability flow cytometry and related antibody-based single cell technologies have become an irreplaceable part of routine clinics and evolved to a powerful tool for exploratory research. Opposed to the intrinsically noisy and sparse data characteristics of most genomic single cell technologies, antibody-based cytometry technologies offer high-resolution measurements of millions of cells across a wide dynamic range facilitating the analysis of large patient cohorts. However, the analysis of multi-cohort studies is often obstructed by batch effects and differences in antibody panels or technology platforms utilized to analyze samples. Here, we present CytoVI, a deep generative model designed for the integration across antibody-based technologies. CytoVI removes technical variation in flow cytometry, CyTOF or CITE-seq data and embeds cells into a meaningful low-dimensional representation corresponding to a cells intrinsic state. CytoVI performs favourable compared to existing tools in data integration tasks, imputes missing markers in experiments with overlapping antibody panels and predicts a cells transcriptome if paired with CITE-seq data. We utilized CytoVI to generate an integrated B cell maturation atlas across 350 proteins from conventional mass cytometry data and automatically detect T cell states associated with disease in a large cohort of Non-hodgkin B cell lymphoma patient measured by flow cytometry. Beyond its applicability for preclinical research, we showcased that CytoVI can automatically identify tumor cells in chronic lymphatic leukemia patients via transfer learning and predict a patient's diagnosis in a fully automated fashion. Therefore, CytoVI represents a powerful deep learning tool for preclinical research and enables an accurate automated analysis of immunophenotypes in patient samples in clinical settings.

1:00 pm – 2:00 pm Lunch break & networking

2:00 pm – 2:40 pm Invited talk by Denis Schapiro, Heidelberg, Germany

Chair: Bertram Bengsch & Henrik Mei

From oncology to cardiology: Spatial omics technologies for topographic biomarker discovery

With improvements in speed and amount of data that can be collected from tissues, data processing and analysis have become the major challenge for spatial biology. Therefore, we developed the open-source histology topography cytometry analysis toolbox (histoCAT), which became the first software specifically tailored to analyse highly multiplexed images. HistoCAT includes advanced machine learning approaches and basic statistical methods integrated in an interactive desktop application. Recently, we have developed a scalable and modular computational pipeline (MCMICRO) enabling the analysis of a variety of highly multiplexed spatial technologies for proteomics (antibody-based) and transcriptomics. We demonstrated the use of MCMICRO on dozens of tissue and tumor images acquired using multiple imaging platforms, thereby providing a solid foundation for the continued development of tissue imaging software. In the second part of my talk, I will cover the Minimum Information about Highly Multiplexed Tissue Imaging (MITI) standard as well as our spatial power analysis framework to improve experimental design strategies. Lastly, I will highlight how we utilized the developed tools to process data with a focus on myocardial infarction and how we are currently building a translational center for spatial profiling (Translational Spatial Profiling Center – TSPC).

2:40 pm – 3:00 pm Farewell & Award Ceremony

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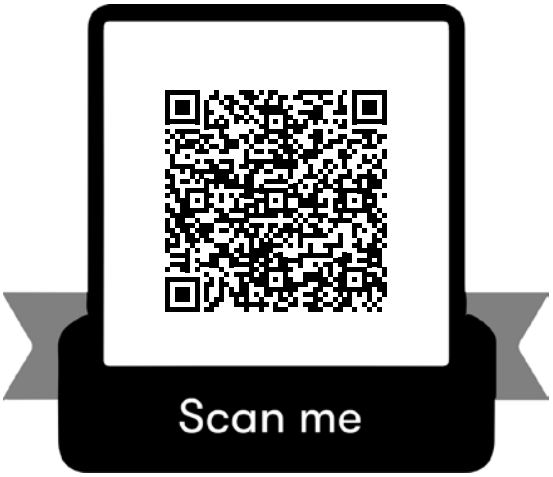

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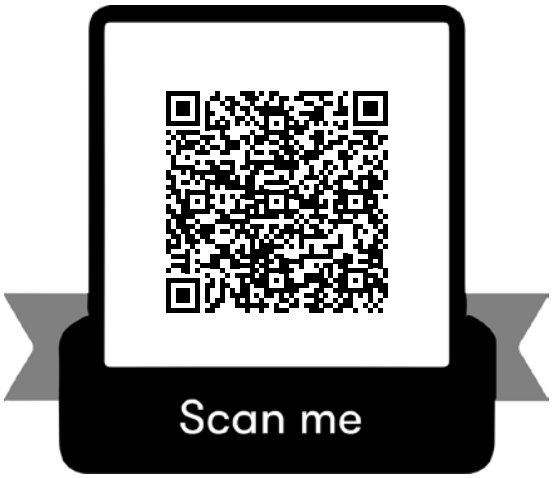

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