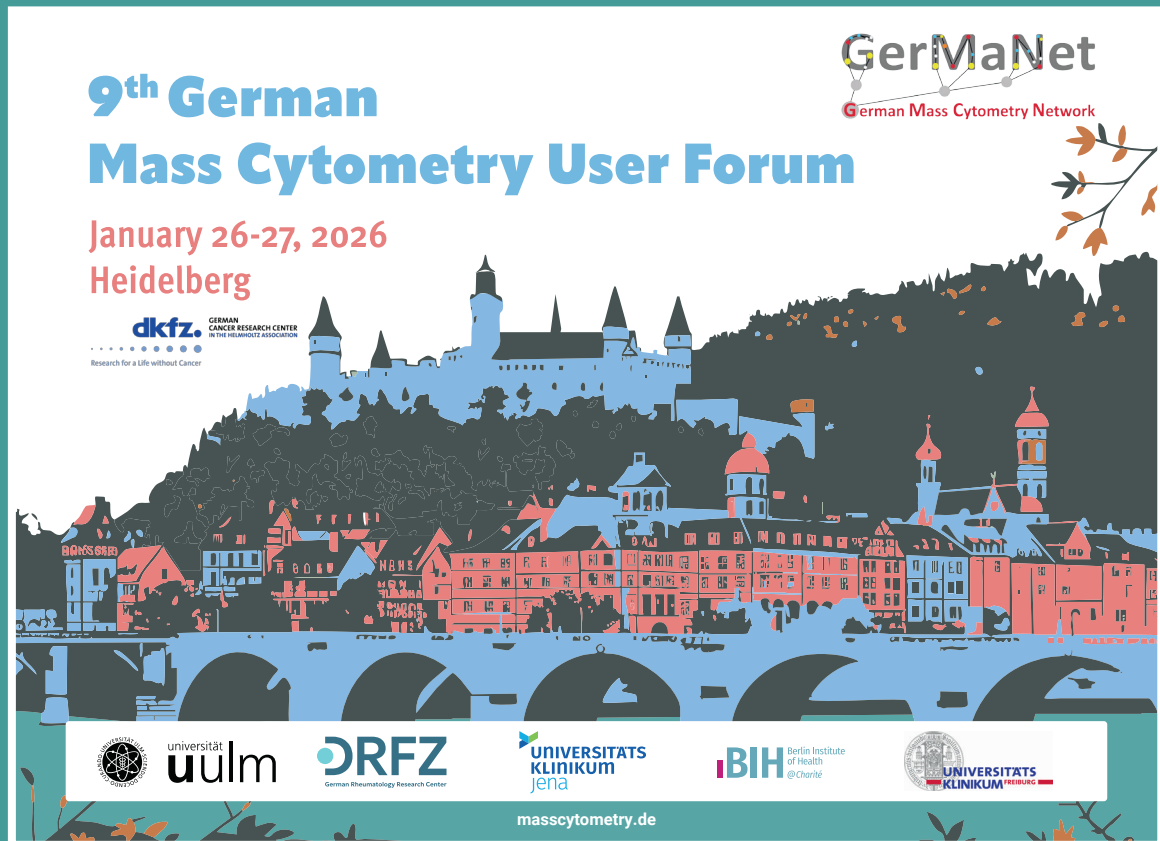Program and Abstract Book

9ᵗʰ German Mass Cytometry User Forum

January 26-27, 2026

Location
DKFZ Heidelberg
Im Neuenheimer Feld 280
69120 Heidelberg

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Website
masscytometry.de

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Dear friends of mass cytometry,

Welcome to the 9th German Mass Cytometry User Forum (GMCUF) which is being hosted this time by the German Cancer Research Center (DKFZ) in Heidelberg.

The GMCUF is more than just a conference — it's a vibrant community gathering where mass cytometry enthusiasts come together to inspire each other, spark new ideas, and push the boundaries of what mass cytometry can achieve. Whether you are an experienced user, a newcomer, or somewhere in between, this meeting offers a unique chance to explore the latest advances, troubleshoot challenges, and build lasting collaborations.

The 2026 program continues the successful format from previous years: an engaging poster session showcasing cutting-edge research and insightful "News from" sessions sharing developments from mass cytometry and imaging sites aacross Germany, Austria and Luxembourg.

We look forward to two inspiring days of discussions, knowledge sharing, and networking.

The organizers of the GMCUF

Sabine Baumgart, Jena
Bertram Bengsch, Freiburg
Désrée Kunkel, Berlin
Sarah Warth, Ulm
Axel Schulz, Berlin
Aoife Gahlawat, Heidelberg
Lena Müller, Vienna
Claudia Peitzsch, Dresden
Ute Hoffmann, Berlin
Jacqueline Hirscher, Berlin

Monday 26.01.2026

8:30 am   Start of registration

9:00 am   Pre-Conference Tutorial - Getting started for Beginners

Chair: Sarah Warth, Ulm, Germany

Désirée Kunkel, Berlin; Claudia Peitzsch, Dresden; Felix Röttele, Freiburg; Axel Schulz, Berlin

11:00 am   Coffee break

11:30 am - 12:00 pm   Opening by the meeting organizers

Chairs: Aoife Gahlawat & Denis Schapiro

Welcome by: Aoife Gahlawat, Heidelberg, Sabine Baumgart, Jena, Bertram Bengsch, Freiburg, Désireé Kunkel, Berlin, Axel Schulz, Berlin, Sarah Warth, Ulm

Welcome talk by Denis Schapiro

Institute for Computational Biomedicine and Institute of Pathology, Heidelberg University Hospital

Quantitative tissue analysis at the Translational Spatial Profiling Center (TSPC)

12:00 pm - 12:40 pm   Invited talk by Sara di Biasi, Modena, Italy

Chairs: Aoife Gahlawat & Denis Schapiro

Dissecting immunometabolic crosstalk between B Cells and T Cells in NSCLC

12:40 pm – 1:10 pm   Techno bites

Chairs: Sarah Warth, Ulm University, & Axel Schulz, DRFZ Berlin

Core Quantum Technologies, Polaris Biology

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

2:30 pm – 3:45 pm   Imaging MC & Data Analysis Session

Chairs: Bertram Bengsch, Freiburg & Désireé Kunkel, Berlin

Invited Talk by by Laurent Gorvel, Marseille, France

Immunomonitoring of cervical tumors: identifying tertiary lymphoid structures and associated immune infiltrate as prognosis markers

Short Talk by Yuan Suo, Freiburg, Germany

Imaging mass cytometry enables spatial chromatin modification profiling at single-cell level

Short Talk by Akhiya Anilkumar Rekha, Lyon, France

AltraFlowSOM: A Semi-Supervised Framework for Scalable Phenotyping of Imaging Mass Cytometry Data

Short talk by Kilian Merz, Heidelberg, Germany

Metabolic reprogramming of neutrophils in colorectal cancer

3:45 pm - 4:15 pm   Coffee break

4:15 pm - 6:00 pm   News from … Part 1

Chairs: Sabine Baumgart, Jena & Axel Schulz, Berlin

News from Heidelberg (DKFZ): Miray Cetin

Therapy-induced metabolic remodeling in HER2-negative breast cancer

News from Dresden: Nisarg Jagdishbhai Dobaria

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

News from Erlangen: Carlo Tur

Lymphatic tissue remodelling following CD19 CAR T-cell therapy assessed by imaging mass cytometry

News from Berlin (MPI-MOLGEN): Marie-Laure Yaspo

News from Berlin (BIH): Désireé Kunkel

New adventures - changing from Hyperion 'Tangerine' to XTi 'Blanche'

News from Vienna: Klara Brozova

Integrative spatial proteomics by Imaging Mass Cytometry and MALDI-MSI uncovers tumor- and microenvironment-driven heterogeneity in breast cancer.

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

Tuesday, 27.01.2026

9:00 am – 9:30 am   Product feature talks by Standard BioTools

Chair: Aoife Gahlawat, Heidelberg

Cheuk Ting Wu, Institute of Anatomy, University of Bern, Switzerland

Imaging Mass Cytometry as a powerful platform to map dynamic liver repair after spatially fractionated radiotherapy (SFRT)

Jayden Gittens, University of Liverpool, UK

Characterising the tumour microenvironment of liver metastases with imaging mass cytometry

9:30 am – 10:45 am   Suspension MC Session

Chairs: Sabine Baumgart, Jena & Sarah Warth, Ulm

Invited Talk by Olivier Molendi-Coste, Lille, France

Batch Effects Correction in Mass Cytometry Data

Short Talk by Mehmet Serdar Koca, Granada, Spain

Center Harmonization for Multicenter CyTOF Immune Monitoring

Short talk by Lucía Rodríguez Doña, Granada, Spain

What it takes to CyTOF-profile 2,500 clinical samples across four immune-mediated diseases

Short Talk by Niclas Schierloh, Freiburg, Germany

SceniTOF - Functional single-cell multiplexed metabolic profiling to map bioenergetics of heteroegeneous immune cells by mass cytometry in mice and men

10:45 am – 11:30 am   Coffee break

11:30 am – 1:00 pm   News from . . . Part 2

Chairs: Claudia Peitzsch, Dresden & Lena Müller, Vienna

News from Ulm: Dominik Schlotter

Multiparametric mass cytometry (CyTOF) for exploring the sex-dependent crosstalk between DNA damage and inflammatory response during aging

News from Berlin (DRFZ): Axel Schulz

Comparative Performance Benchmarking of Lunarion vs. Helios and CyTOF XT Mass Cytometers

News from Luxembourg: Antonio Cosma

Democratization of mass cytometry and data analysis (#BI4Flow)

News from Halle: Marcus Bauer

Establishment of Imaging Mass Cytometry for High-Dimensional Spatial Profiling of Decalcified Bone Marrow Biopsies

News from Jena: Sabine Baumgart

Introducing UriTOF: A Preservation Workflow for Urine CyTOF

News from Salzburg: Markus Steiner & Stephan Drothler

Interactive Cell Gating and ultra-specific detection of RNA: New IMC tools for focused microenvironment analysis

News from Cologne: Daniel Bachurski

From TeLEV to MULTI-TeLEV – Simultaneous profiling of EV uptake and recipient cell signaling from six EV sources

1:15 pm – 1:55 pm   Invited talk by Marcin Poręba, Wrocław

Chairs: Hyun-Dong Chang, Berlin & Bertram Bengsch, Freiburg

The application of chemical probes for imaging protease and kinase activity in tumor cells by mass cytometry

1:55 pm – 2:15 pm   Farewell & Award Ceremony

2:15 pm – 3:00 pm   Lunch to go

3:00 pm – 4:00 pm   Inaugural Assembly of the Mass Cytometry working group

Arbeitskreis Massenzytometrie under the umbrella of the German Society for Cytometry (DGfZ)

Monday 26.01.2026

9:00 am - 11:00 am Pre-Conference Tutorial - Getting started for Beginners

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

Désirée Kunkel, BIH Cytometry Core Facility, Berlin Institute of Health (BIH) at Charite - Universitätsmedizin Berlin, Berlin, Germany

Claudia Peitzsch, Mass Cytometry Facility, Center for Regenerative Therapies Dresden (CRTD), Dresden University of Technology, Dresden, Germany

Felix Röttele, Department of Medicine II, Medical Center – University of Freiburg, Faculty of Medicine, University of Freiburg, Germany

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

Getting started – An Introduction to Mass Cytometry

This tutorial provides a comprehensive, practice-oriented introduction to mass cytometry for both suspension and imaging applications for beginners.

Our five experts will explain the principles of mass cytometry, demonstrate how it is applied to cell suspensions and tissue sections, and walk you through typical experimental workflows. You will gain hands-on insight into critical practical steps, including metal conjugation, sample barcoding, spillover correction, and strategies for minimizing batch effects and normalizing data across runs. A dedicated segment will highlight Imaging Mass Cytometry (IMC), outlining its key advantages and guiding you through the process of designing and establishing multiplexed antibody panels. 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.

| 11:00 am – 11:30 am	Coffee break

11:30 am   Welcome to the 9th GMCUF

Chairs: Aoife Gahlawat, Heidelberg & Denis Schapiro, Heidelberg

Welcome by: Aoife Gahlawat, Sabine Baumgart, Bertram Bengsch, Désireé Kunkel, Axel Schulz, Sarah Warth

Welcome talk by Denis Schapiro

Institute for Computational Biomedicine and Institute of Pathology, Heidelberg University Hospital

Quantitative tissue analysis at the Translational Spatial Profiling Center (TSPC)
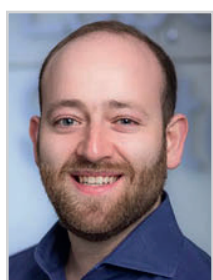
In this talk, I will introduce our recently founded Translational Spatial Profiling Center (TSPC) and the computational tools we are developing to enable robust and high-throughput analysis of a variety of highly multiplexed spatial technologies for proteomics (antibody-based) and transcriptomics. I will also introduce our spatial power analysis framework for improved experimental design strategies and for neighborhood method benchmarking. Finally, I will highlight how we utilized the developed tools to process data with use cases in oncology and cardiology.

12:00 pm - 12:40 pm   Invited talk by Sara De Biasi

University of Modena and Reggio Emilia, Department of Medical and Surgical Sciences for Children & Adults, Modena, Italy

Chairs: Aoife Gahlawat, Heidelberg & Denis Schapiro, Heidelberg

Dissecting immunometabolic crosstalk between B Cells and T Cells in NSCLC

B cells have emerged as key components of the tumor microenvironment (TME) in non-small cell lung cancer (NSCLC). Despite increasing evidence supporting their role in antitumor immunity, limited information is available regarding the phenotypic diversity, metabolic features, and functional interactions of B cells with T cells in NSCLC. In this study, we comprehensively characterized B cell populations within the NSCLC TME using polychromatic flow cytometry, mass cytometry, and spatial transcriptomics. Our analyses identified multiple intratumoral B cell clusters exhibiting distinct metabolic and functional properties, including a population of VISTA-expressing regulatory B cells (Bregs). Targeted liquid chromatography–tandem mass spectrometry confirmed VISTA expression on B cells. VISTA$^+$ Bregs displayed high bioenergetic demand and produced a broad spectrum of cytokines, including interleukin (IL)-10, transforming growth factor (TGF)-β, IL-6, tumor necrosis factor (TNF), and granulocyte–macrophage colony-stimulating factor (GM-CSF). Spatial transcriptomic analyses revealed close colocalization of B cells with CD4$^+$ and CD8$^+$ T lymphocytes within the TME. Computational inference of intercellular communication using NicheNet predicted functional B–T cell interactions mediated through the VISTA–PSGL-1 signaling axis. Consistent with these predictions, spatial proximity analyses demonstrated that PSGL-1–expressing T cells are frequently adjacent to VISTA$^+$ B cells in tumor tissues. Notably, tumor-infiltrating CD8$^+$ T cells expressing PSGL-1 exhibited enhanced metabolic activity. Together, these findings uncover a metabolically active, immunoregulatory B cell compartment in NSCLC and highlight a potential VISTA-mediated mechanism of B–T cell crosstalk within the TME.

Techno Bites

Chair: Sarah Warth, Ulm University, & Axel Schulz, DRFZ Berlin

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

2:30 pm - 3:45 pm Imaging MC & Data Analysis Session

Chairs: Bertram Bengsch, Freiburg & Désireé Kunkel, Berlin

Invited Talk by Laurent Gorvel

Marseille Cancer Research Center, Marseille, France

Immunomonitoring of cervical tumors: identifying tertiary lymphoid structures and associated immune infiltrate as prognosis markers

Cervical tumors are usually treated using surgery, chemotherapy, and radiotherapy and would benefit from immunotherapies. However, the immune microenvironment in cervical cancer remains poorly described. Tertiary lymphoid structures (TLS) were recently described as markers for better immunotherapy response and overall better prognosis in patients with cancer. We evaluated the cervical tumor immune microenvironment, specifically focusing on TLS, using combined high throughput phenotyping, soluble factor concentration dosage in the tumor microenvironment, and spatial interaction analyses.

We found that TLS presence was associated with a more inflammatory soluble microenvironment, with the presence of B cells as well as more activated macrophages and dendritic cells (DC). Furthermore, this myeloid cell activation was associated with the expression of immune checkpoints, such as PD-L1 and CD40, and the proximity of activated conventional type 2 DCs to CD8+ T cells, indicating better immune interactions and tumor control. Finally, we associated TLS presence, greater B-cell density, and activated DC density with improved progression-free survival, substantiating TLS presence as a potential prognostic marker. Our results provide evidence that TLS presence denotes cell activation and immunotherapy target expression.

Short Talk by Yuan Suo

Clinic for Internal Medicine II, University Medical Center Freiburg, Germany.

Imaging mass cytometry enables spatial chromatin modification profiling at single-cell level

Introduction: Chromatin modification plays a crucial role in tumor development by regulating gene activation and repression. Current approaches primarily rely on sequencing-based methods that assess single modification and often lack spatial information. In this project, we employed imaging mass cytometry (IMC) to spatially characterize multiple chromatin modification profiles at the single-cell level in human liver and brain tissue.

Methods: We profiled human liver tissue with a 42-antibody panel including immune, stromal, and parenchymal markers with 11 chromatin modification markers. After image segmentation, single-cell data were extracted to quantify marker expression and to define cellular and epigenetic heterogeneity.

Results: We identified 17 cell types in the human liver based on lineage marker expression, as well as 14 distinct epigenetic states reflecting different levels of gene activation and repression. Within each cell type different epigenetic states coexisted, while certain states were preferentially associated with specific cell types.

Spatial neighborhood analysis classified the tissue into tumor-enriched, hepatocyte-enriched, central vein−associated, immune-enriched, and immune−stroma−enriched neighbourhoods. Clustering based on epigenetic states composition further defined three "Epi-niches", which enriched for different chromatin modification states. Correlation analysis showed that hepatocyte-enriched and central vein−associated neighbourhood correlated to Epi-niche 3, and tumor-enriched regions with Epi-niche 1 while immune-enriched, and immune−stroma−enriched neighbourhood with Epi-niche 1 and 2.

Epigenetic state composition of neighboring cells (K=10) varied by cell type. For example, CD8+ T cells were most frequently surrounded by cells in epigenetic state 4. And within the tissue, CD8+ T cells interacted significantly with CD4+ T cells and Iba1+ macrophages. Consistently, epigenetic state 4 was associated with CD4+ T cells, Iba1+ macrophages, and CD8+ T cells, suggesting shared chromatin modification features within these interacting cells.

Conclusion: in summary, we established the methods of chromatin modification profiling using imaging mass cytometry. Our results indicate that cellular epigenetic states are spatially organized and are shaped by local cell−cell interactions within the tissue microenvironment.

Short Talk by Akhiya Anilkumar Rekha

Lymphocytes B, Autoimmunité et Immunothérapies – LBAI (UMR 1227), Université de Bretagne-Occidentale
AltraBio SAS, Lyon, France

AltraFlowSOM: A Semi-Supervised Framework for Scalable Phenotyping of Imaging Mass Cytometry Data

Background: Imaging Mass Cytometry (IMC) enables high-dimensional spatial profiling of complex tissue microenvironments, yet current analysis pipelines still rely heavily on unsupervised clustering followed by manual post-hoc annotation. While expert annotation is essential for assigning biological meaningful populations, the upstream clustering step is performed without biological priors and can be strongly influenced by technical variation, leading to clusters that are not immediately biologically interpretable. This workflow is labour-intensive, difficult to scale, and becomes increasingly challenging in large multi-sample datasets, where batch effects and data heterogeneity can obscure rare but biologically important cell populations and reduce the biological meaningfulness of clustering.

Objectives: Develop and evaluate a semi-supervised clustering tool that better aligns with the biological knowledge that scales with larger datasets, possibly reducing the cons of classical workflow.

Methods: We developed AltraFlowSOM, a semi-supervised extension of the FlowSOM framework. AltraFlowSOM extends the FlowSOM framework by incorporating Supervised Self Organizing Maps (SSOM's) an existing supervised extension of SOM to integrate partial expert annotations directly into the clustering process, thereby guiding the formation of clusters toward biologically meaningful populations. The framework merges raw marker expression with biological knowledge layers (e.g., manual gating labels) using a multi-layer SSOM architecture. This guides cluster formation toward biologically relevant phenotypes while preserving the ability to discover previously unrecognized populations.

We benchmarked the performances across major analytical strategies, including:

- Unsupervised (FlowSOM & Phenograph, with and without batch-effect correction)
- Supervised learning (Random Forest)
- AltraFlowSOM (semi-supervised)

Two independent IMC datasets were used for evaluation; Lupus Nephritis (n=22) and Sjögren's Disease (n=10).

Results: AltraFlowSOM achieved the highest concordance with manually gated ground truth, outperforming FlowSOM, Phenograph (with and without batch correction), and Random Forest across Adjusted Rand Index (ARI) and F1-score metrics. High performance persisted even under full supervision and when only partial annotations were available; models trained on only a subset of samples performed comparably to those trained on fully annotated datasets. Using a leave-one-out strategy, training on (n-1) samples and testing on the remaining held-out sample, AltraFlowSOM demonstrated strong generalizability across unseen patient samples and across disease indications. The method improved resolution of rare and biologically meaningful cell populations, and produced phenotypes that aligned more closely with known immunobiology.

Conclusion: AltraFlowSOM integrates expert knowledge directly into cluster formation, bridging the gap between manual gating and automated phenotyping. It enhances biological interpretability, increases sensitivity to rare populations, and provides a scalable, generalizable tool for high-dimensional IMC data. This framework supports reproducible, biologically informed cellular phenotyping in autoimmune disease research and is broadly applicable across spatial and single-cell cytometry platform

Short talk by Kilian Merz

German Cancer Research Center (DKFZ), Heidelberg, Germany

Metabolic reprogramming of neutrophils in colorectal cancer

Neutrophils constitute a major component of the tumor microenvironment and are increasingly recognized as more diverse and long-lived than previously thought. Yet, their specific roles and prognostic significance especially in colorectal cancer remain poorly defined, largely due to challenges in profiling them. Here, we reveal a stage-dependent prognostic effect of neutrophil infiltration in colorectal cancer: beneficial in early-stage disease but detrimental in late-stage disease. Early-stage neutrophils were enriched for inflammatory gene programs, including tumor necrosis factor alpha (TNFα), whereas late-stage neutrophils displayed signatures of metabolic reprogramming. Using spatial proteomics, we identified five distinct neutrophil clusters in human biopsies based on metabolic and phenotypic states. This included "netotic" neutrophils enriched in reactive oxygen species (ROS)-generating enzymes and MMP9-expressing

neutrophils with impaired ROS activity. "Netotic", possibly cytotoxic, neutrophils predominated in early stages, while MMP9-expressing, possibly angiogenic, neutrophils were more frequent in late stages. Late-stage neutrophils appeared metabolically impaired, with reduced engagement of the pentose phosphate pathway essential for ROS production. Spatial analysis identifies that interaction with a highly glycolytic tumor cell niche is associated with the metabolically impaired neutrophils in late stage suggesting metabolic competition as a driver of neutrophil dysfunction.

Together, our findings uncover a stage-dependent prognostic effect of neutrophils in colorectal cancer, linking their functional rewiring to metabolic constraints within the tumor microenvironment.

3:45 pm – 4:15 pm Coffee break

4:15 pm - 6:00 pm News from ... Part I

Chairs: Sabine Baumgart, Jena & Axel Schulz, Belrin

News from Heidelberg (DKFZ): Miray Cetin

Hartmann Lab, DKFZ, Heidelberg, Germany

Therapy-induced metabolic remodeling in HER2-negative breast cancer

Breast cancer remains a leading cause of cancer-related mortality among women. Although it is often diagnosed early, up to 35% of HER2-negative cases relapse as metastatic disease, with median overall survival ranging from 2 years (TNBC) to 5 years (hormone receptor-positive). Several new treatments have been tested in early-stage disease to reduce metastatic relapse, including antiangiogenic agents such as bevacizumab. Bevacizumab can normalize the vasculature and stroma in about one-third of cases, leading to a significant metabolic shift in cancer cells from primarily glycolytic to mitochondrial energy production. This shift can be exploited therapeutically by combining antiangiogenics with mitochondrial inhibitors, known as "metabolic synthetic lethality." Using spatially resolved single-cell proteomics, we investigated samples from a phase 0/I clinical

study (NCT02806817) that combined bevacizumab with ME-344, a mitochondrial complex I inhibitor. We show that bevacizumab reshapes vascular and epithelial metabolism and reorganizes immune cell interactions, thereby increasing epithelial reliance on oxidative phosphorylation. ME-344 then leverages this state to cause mitochondrial dysfunction, reprogram cellular metabolism, and induce oxidative damage in epithelial cells. This is accompanied by an accumulation of iron-rich CD163+ macrophages in certain niches, along with activation and exhaustion of CD8+ T cells. These findings highlight the importance of spatial context in evaluating targeted therapies and suggest that mitochondrial and iron metabolism may offer new opportunities to modulate tumor immunity in combination treatment strategies.

News from Dresden: Nisarg Jagdishbhai Dobaria

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

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

Psoriasis (PsO) and psoriatic arthritis (PsA) involve interconnected pathologies of the skin and joints. While Innate Lymphoid Cells (ILCs) are implicated in the "skin-joint axis," the mechanisms driving the transition from cutaneous to joint inflammation remain unclear. This study utilizes Imaging Mass Cytometry (IMC) to spatially profile immune populations in PsO and PsA lesional skin.

The high-dimensionality of our 33-antibody panel allowed for the simultaneous assessment of broad lineage and functional markers on a single tissue section. This multiplexing capability enabled the precise resolution of heterogeneous populations—including rare ILCs, Tissue-Resident Memory T cells (TRMs), and distinct monocyte and T-cell subsets—while preserving their spatial context within the tissue architecture.

In a pilot analysis of two patient samples, we observed that the chemokine CXCL16 was predominantly expressed in the epidermis and monocytes. Furthermore, preliminary comparisons suggested distinct immunophenotypes: the PsO sample exhibited a trend toward stronger expression of both CXCL16 and its receptor CXCR6, as well as a higher frequency of TRMs, compared to the PsA sample.

These initial observations suggest that the CXCL16/CXCR6 axis may be more prominent in the migration of the immune cells in PsA patients. While validation in a larger cohort is required, this spatially resolved approach demonstrates the feasibility of using IMC to uncover disease-driving factors. Expanding this study will be crucial for identifying biomarkers predictive of the migration of skinderived immune cells to joint tissues.

News from Erlangen: Carlo Tur

Department of Medicine 3 - Rheumatology and Immunology, Friedrich-Alexander-Universität Erlangen-Nürnberg and Uniklinikum Erlangen, Erlangen, Germany.

Deutsches Zentrum für Immuntherapie (DZI), Friedrich-Alexander-Universität Erlangen-Nürnberg and Uniklinikum Erlangen

Lymphatic tissue remodelling following CD19 CAR T-cell therapy assessed by imaging mass cytometry

Anti-CD19 chimeric antigen receptor (CAR) T-cell therapy can induce long-term drug-free remission in patients with autoimmune diseases (AIDs). Two previous proof-of-concept studies based on sequential lymph node biopsies demonstrated a consistent and complete B-cell depletion in lymphatic tissue after CD19 CAR T-cell treatment, as assessed by conventional immunohistochemistry, suggesting a potential mechanism underlying an "immune reset" in AIDs.[1,2]

In this study, we applied imaging mass cytometry (IMC) to deconvolute cellular heterogeneity at single-cell resolution, analyzing 1,328,358 cells to investigate changes in spatial distribution and cellular neighborhoods before and after CAR T-cell therapy, in comparison with healthy controls. In parallel, lymph node samples from patients treated with the standard-of-care B-cell–depleting agent rituximab were analyzed.

We included 15 patients treated with CD19 CAR T cells and 7 patients treated with rituximab, all with paired baseline and 60-day follow-up inguinal needle biopsies, as well as 5 disease-free lymph node samples obtained during surgery from different anatomical sites, serving as healthy controls. In addition, for 8 of the 15 CAR T-cell–treated patients, lymph node samples collected one year after treatment were available.

Stromal, endothelial, B, T and myeloid cell compartments were identified, and immune subpopulations were further characterized, with a particular focus on the follicular network, including germinal center B cells (CD23+Bcl6+), follicular dendritic cells (CD23+CD21+) and T follicular helper cells (CD3+PD-1+Bcl6+). IMC confirmed complete B-cell depletion following CAR T-cell therapy, accompanied by a profound abrogation of the follicular architecture, and demonstrated a full restoration of normal follicular organization

one year after treatment. Notably, early follow-up biopsies revealed, through neighborhood analysis, repopulation of the ablated follicular areas by stromal and myeloid cells, together with an increased presence of Foxp3^{+} cells. By employing IMC, we provide an in-depth characterization of the lymph node immune landscape and its dynamic remodeling following CD19 CAR T-cell therapy. Our findings not only corroborate previous evidence of profound and durable B-cell depletion, but also uncover previously unappreciated alterations in T-cell and myeloid cell responses within a fully B-cell-depleted environment.

News from Berlin (MPI-MOLGEN): Marie-Laure Yaspo

News from Berlin (BIH): Désireé Kunkel

BIH Cytometry Core Facility, BIH at Charite - Universitätsmedizin Berlin, Berlin, Germany

New adventures - changing from Hyperion 'Tangerine' to XTi 'Blanche'

This talk will highlight the challenges - and positive surprises - we have encountered at the BIH CCF during our transition from the CyTOF2/Helios/Hyperion system to the new CyTOF XT/XTi platform. A particular focus will be on the core facility's perspective, including our efforts to set up workflows for service requests, sample intake, sample measurements, and data management.

News from Vienna: Klara Brozova

Medical University of Vienna, Vienna Austria

Integrative spatial proteomics by Imaging Mass Cytometry and MALDI-MSI uncovers tumor- and microenvironment-driven heterogeneity in breast cancer.

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 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 Freiburg: Bertram Bengsch
Klinik für Innere Medizin II, Universitätsklinikum Freiburg, Freiburg, Germany

Heavy metal based research in Freiburg: Update 2026

This talk will give a brief overview about recent developments in the mass cytometry unit Freiburg and will discuss an approach to understand comprehensive signaling network changes in primary human exhausted T cells at the single-cell level.

6:00 pm - 10:00 pm Poster Session

Evaluation team: Désireé Kunkel & Sarah Warth

Adrián Barreno Sánchez

Mass Cytometry Analysis Defines Clusters of SLE Patients with Differential Mucosal Phenotypes that Correlate with Immune Activation and Disease Severity

Adrián Barreno Sánchez[1,2], Alissa Karina Yudiputri[1], Heike Hirseland[1], Anne Beenken[1,4], Paulina Rybakowska[3], Concepción Marañón Lizana[3], Marta E. Alarcon Riquelme[3,5], Tobias Alexander[1,4], Henrik E. Mei[1], Axel R. Schulz[1], Hyun-Dong Chang[1,2]

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

2. Department for Cytometry, Institute of Biotechnology, Technische Universität Berlin, Berlin, Germany

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

4. Department of Rheumatology and Clinical Immunology, Charité Universitätsmedizin Berlin, Berlin, Germany

5. Institute for Environmental Medicine, Karolinska Institutet, Stockholm, Sweden

Keywords: Systemic Lupus Erythematosus, mucosal immunity, mass cytometry

Purpose: Systemic lupus erythematosus (SLE) is a prototypical systemic autoimmune disease characterized by immune tolerance breakdown and the production of autoantibodies. Although the precise mechanisms underlying individual clinical manifestations and prognosis in SLE are poorly understood, recent evidence revealed a dysregulation of mucosa-related immune populations in SLE. This highlights the need to investigate mucosa-related immunity as a potential source of patient heterogeneity.

Methods: To explore the role of mucosa-related immunity in SLE heterogeneity, we recently established a robust and scalable mass cytometry workflow for deep immune cell phenotyping and ex-vivo phosphorylation profiling of peripheral blood with two >50-plex antibody panels. We used a semi-automated data pre-processing and analysis pipeline to identify main immune lineages and subpopulations, and performed unsupervised clustering of patients based in the abundance of immune populations with a potential mucosal origin. Supervised analysis was performed to compare immune phenotypes and activation across patient groups and correlate them to clinical parameters

Results: We identified multiple lymphoid populations with a potential mucosal association present across SLE patients and healthy donors: namely IgA+ plasmablasts, a4b7+ CD4T cells, aEb7+ CD8T cells, mucosa-associated invariant T (MAIT) cells, gd T cells, and other subpopulations of double negative (DN) T cells. Next, we stratified SLE patients in 3 clusters with differential abundance of mucosal populations. Cluster 1 is primarily defined by an increased abundance of DN T cells and exhausted CD27- gdT cells, while cluster 3 is mainly driven by an increased abundance of a4b7+ CD4T and aEb7+ CD8T cells. As compared to cluster 1, patients belonging to cluster 3 show an elevated abundance of CD38+ CD21- DN B cells with increased pSTAT5 signaling, higher pSTAT3/5 signaling in naïve and memory CD4 and CD8 T cells and an increased abundance of cytotoxic Slamf7+ CD56high NK cells. In the myeloid compartment, patients from cluster 3 showed increased phospho-signaling activity in monocytes and neutrophils (pCREB, pMAPK and pSTAT1). Clinically, patients in cluster 3 also showed an increased prevalence of renal involvement and higher SLEDAI-2K score and Physician Global Assessment.

Conclusion: Here, we describe differential mucosal signatures in SLE patients that correlate with immune activation, clinical manifestation and disease severity. Further work is required to elucidate the link between the changes observed in peripheral blood and potential alterations in the mucosal tissue immunity. To assess this, I will integrate blood phenotypes with microbiota phenotyping and sequencing data and correlate it with other paired data modalities (e.g. serum proteomics, stool metabolomics). Overall, our work suggests that mucosal immune dysregulation might be a relevant source of patient heterogeneity in SLE.

Jürgen Beck

RNAscope mediated RNA detection by IMC

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Classical IMC is confined to detection of protein markers using metal-labeled antibodies. Recently the range of Hyperion-detectable macromolecules has been expanded to RNA by combining advanced in situ hybridization techniques with metal-labeled oligonucleotide probes. This novel technique promises sensitive sequence-specific detection of cellular, viral or bacterial RNAs with high spatial resolution, which can be combined with classical antibody staining e.g. for identification of virus replicating or chemokine producing cells.

We have started to establish Hepatitis B Virus (HBV) RNA detection in our lab using the „RNAscope HiPlex-IMC" system (Advanced Cell Diagnostics). RNAscope, primarily developed for fluorescent RNA detection, can be adapted to IMC by conjugation of metals to sulfhydryl-modified oligonucleotides using the Standard Biotools' MaxPar Kit.

To test the performance of the assay we used human hepatoma cell lines transiently or stably transfected *in vitro* with HBV. FFPE samples were generated by formalin fixation and paraffine embedding of cultured cells. FFPE sections were stained for HBV RNA by sequential hybridization with (i) a HBV specific probe, (ii) signal amplifier probes and (iii) an orthogonal metal-labeled imaging probe, optionally followed by antibody staining. Specificity was assessed using naive cells and control probes.

Images aquired with the Hyperion system revealed HBV probe dependent signal accumulation in cells from HBV replicating samples. Our data demonstrate proof-of-principle for IMC-mediated sequence-specific RNA detection and provide a basis for future implementation of RNAscope into our IMC analysis pipeline.

Vera Bockhorn & Sebastian Ferrara

A Metadata Model and Validation Framework for Immunological Data within NFDI4Immuno: Building a Foundation for Comprehensive Data Integration, Analysis, and Open Science

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The rapid advancement of single-cell technologies— including flow cytometry, mass cytometry, and single-cell sequencing—has enabled the generation of increasingly information-rich datasets, intensifying the need for robust data sharing and reuse within immunological research.

The National Research Data Infrastructure for Immunology (NFDI4Immuno) addresses this need by integrating immunological data and metadata. By harmonizing metadata standards, ontologies, data models, and programmatic interfaces in collaboration with other NFDI consortia, NFDI4Immuno promotes seamless discovery and cross-referencing of data. Central to the initiative is the implementation of data stewardship practices aligned with the FAIR principles and the support of user communities in leveraging these resources effectively.

The project establishes a federated network of institutional repositories and accompanying software pipelines for standardized deposition,

annotation, and retrieval of immunological datasets, while ensuring proper access control and provenance tracking. This addresses current barriers caused by inconsistent sharing and annotation practices, mitigating data redundancy and enabling more comprehensive analysis across studies.

Here, we present the current state of our metadata model, defining a comprehensive metadata structure compliant with the General Data Protection Regulation (GDPR) that includes controlled vocabularies to enforce standardized data annotation. Our validation and submission process entails comprehensive quality checks of submitted metadata, meaningful error reporting, and efficient data file upload.

Ultimately, NFDI4Immuno seeks to foster a culture of Open Science and reproducibility, empowering the immunological community to derive deeper insights and accelerate research progress.

Chotima Böttcher

Immune-proteo-metabolomic changes link to Aβ and tau pathology in Alzheimer disease

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INTRODUCTION: Tryptophan metabolism is increasingly implicated in Alzheimer's disease (AD), particularly through catabolites acting as aryl hydrocarbon receptor (AhR) ligands that influence neuroinflammation. However, their relationships with core AD pathology-amyloid-β (A) and tau (T) deposition-and associated immune-proteomic alterations remain unclear.

METHODS: We performed integrative multi-omics/high-dimensional profiling of cerebrospinal fluid (CSF) and peripheral blood from A-T- (n=19) and A+T+ (n=35) individuals using targeted metabolomics, mass cytometry, and NULISA-based proteomics, alongside inter-compartmental correlation analysis. Brain-derived tryptophan catabolism was investigated using single-nucleus RNA sequencing (snRNA-seq).

RESULTS: Thirteen differentially expressed CSF proteins in A+T+ individuals correlated positively with tryptophan metabolites and pyroglutamate, and negatively with regulatory T cells, isobutyrate and dendritic cells. Similar patterns were observed in blood. snRNA-seq suggested partial brain origin of metabolites.

DISCUSSION: Our findings highlight conserved immune-metabolic-proteomic signatures in AD and implicate tryptophan metabolism as a cross-compartmental factor relevant for biomarker and therapeutic development.

Martin Borgmann

Characterizing the Drivers of Gastroesophageal Adenocarcinoma Development using the B6.IL-1beta(EBV) tcw mouse model

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Gastroesophageal adenocarcinoma (GEAC) is a malignancy of the distal esophagus at the squamocolumnar junction. Most GEAC patients have a history of Barret's esophagus (BE), a metaplastic adaptation to chronic reflux. BE is initially caused by inflammation and, in rare cases, can progress over a dysplastic intermediate state to GEAC. Besides genetic alterations, changes in the microenvironment are key drivers in enabling disease progression. To study these, we use B6.IL-1beta(EBV)tcw mice, which is a well-characterized model of GEAC, with dysplastic lesions developing at the squamocolumnar junction of the gastric cardia. This model offers unique opportunities to study dynamic interactions between the immune system, stroma, and epithelial transitions over time. Our goal is to identify factors that drive progression from BE to GEAC to enhance patient care by tailoring preventive measures. A total of 198 formalin-fixed, paraffin-embedded (FFPE) gastric cardia tissues from B6.IL-1beta(EBV)tcw mice aged 4 to 12 months, covering all disease stages and various experimental conditions (aggravating and ameliorating), were combined into tissue microarrays. Imaging mass cytometry enabled detailed, spatially resolved proteomic profiling of cell types and activation states. For image pre-processing we followed the Bodenmiller-Steinbock pipeline, and advanced single-cell and spatial analyses were performed using R and Python. In standard PL2 mice, the metaplastic marker TFF2 declined with age and was further reduced by NSAID treatment. Stathmin 1, a microtubule destabilizer linked to cancer progression, also decreased. Markers of immune infiltration (CD45), regulatory T cells (Foxp3), and mesenchymal cells (Vimentin) increased with age. E-cadherin expression declined, indicating epithelial disruption. Fibronectin was elevated in high-fat diet mice, coinciding with earlier biomarker shifts. INOS remained consistently higher across ages, while pYAP expression decreased over time. These data highlight age- and diet-related changes in immune and stromal components within the gastric cardia microenvironment, alongside reductions in metaplastic (TFF2) and cancer-associated markers (Stathmin1), particularly under NSAID treatment. Together, these findings reflect progressive remodeling that underpins GEAC development in the PL2 model.

Natalia Ćwilichowska-Puślecka

Application of metal-tagged TOF probes for proteases in breast cancer using imaging mass cytometry

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Proteases are known to be among the key players in modulating the tumor microenvironment. Therefore, to gain a better insight into such a complex cellular landscape, we decided to use mass cytometry, which uses antibodies conjugated with stable metal isotopes. The excellence of this method lies in the fact that each isotope has its peak on the mass spectrum, eliminating signal overlap and consequently enabling the monitoring of more than 50 parameters with a single cell resolution. Mass cytometry enables

the analysis of cells in suspension and as a tissue imaging system - Imaging Mass Cytometry (IMC), providing comprehensive information about tissue architecture and its microenvironment. Since antibodies allow detection of the total amount of enzymes, we postulate that this approach requires a more sophisticated attitude, like using activity-based probes (ABPs). The undoubted advantage of using ABPs is the detection of only active enzymes. In our laboratory, we are adapting ABPs for mass cytometry purposes. The first demonstration of the technology was successfully conducted, using PBMC from a healthy donor [1]. We are now trying to extend the technology to use the same probes in IMC. In this study, we use metal-labeled antibodies for cancer architecture along with protease antibodies, enzymes inhibitors and corresponding ABPs to stain breast cancer tissues. This approach allows us to detect the localization and activity pattern of selected cancer-associated proteases. Our approach might bring many benefits to the field of personalized cancer diagnosis and treatment, as proteases contribute to anticancer chemo- and immunotherapy resistance. Moreover, our approach might be directly used for the development of highly selective antibody-drug conjugates that are activated by proteases displaying elevated activity.

[1] Poreba, Marcin et al. "Multiplexed Probing of Proteolytic Enzymes Using Mass Cytometry-Compatible Activity-Based Probes." Journal of the American Chemical Society vol. 142,39 (2020)

Lisa-Marie Dieckmann

TAhRget: Dietary Modulation of AhR-mediated Immune Phenotypes in Healthy Individuals

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The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that interacts with environmental contaminants as well as with nutritional ligands. The AhR is known to be involved in several inflammatory processes and immune system dysregulation like chronic kidney disease (CKD) and multiple sclerosis (MS), but a deeper understanding of how nutrition modulates AhR-mediated immune responses is needed.

Plant-based diets, rich in tryptophan, can activate AhR, affecting its role in immune responses.

To better understand the nutritional effect on AhR-related inflammatory responses, 12 healthy volunteers were enrolled to a 3-day contrasting dietary challenge. Six volunteers in each diet group were exposed to a western diet containing pro-inflammatory nutrients (saturated fatty acids, refined grains, sugars) or plant-based diet, containing anti-inflammatory nutrients (omega-3 fatty acids, fiber, polyphenols). Volunteers were kept in a highly controlled inpatient setting (German Aerospace Center) to reduce environmental influences. For immune phenotyping, whole blood samples were analyzed using CyTOF. Three different antibody panels were applied to target T cells, B cells and granulocytes. Overall findings imply that plant-based diet might positively influence AhR-mediated inflammatory responses. Cluster frequencies revealed an increase in CD33+CD16+ granulocytic myeloid-derived suppressor cells (GMDSCs) in plant-based diet compared to western diet.

Unravelling the interaction between AhR, diet, microbiome and health could be the fundament for personalized dietary interventions and provide strategies to prevent chronic inflammatory diseases such as CKD or MS.

Benjamin Dorschner

High-Dimensional Immune Profiling Identifies Cellular Correlates of SARS-CoV-2 Non-Transmission in Households

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Identifying host immune features that reduce transmission of respiratory viral infections remains challenging. Using SARS-CoV-2 as a model pathogen, the COVID-19 pandemic provided an opportunity to investigate such factors within households where close proximity is given and transmission probability therefore high. We applied high-dimensional mass cytometry to characterize immune profiles of cryopreserved PBMCs from 72 individuals across 22 families with well-documented infection status confirmed by nasal PCR and/or antigen testing. PBMCs were stimulated overnight with peptide pools derived from SARS-CoV-2, endemic coronavirus NL63, or human actin (control), and analyzed using a 37-parameter CyTOF panel capturing major immune subsets and cytokine responses. To identify immune correlates of reduced transmission probability, we employed an adaptive elastic-net model integrating cell frequencies and cytokine-production profiles.

The model selected four cell types and functional states—IFNγ expression of CD4+CD161+ effector memory Th2 cells, frequency of CD8+CD25+ effector memory T cells, CD57+ NK cells and double negative T cells. Each predictor reflects an effector-memory or cytotoxic immune state that has been associated with rapid viral control. All predictors were specific for transmission probability, as there was no overlap with predictors of infection status. IFNγ expression of CD4+CD161+ effector memory Th2 cells exhibited the strongest correlation with SARS-CoV-2 non-transmission. This finding points towards a potential role of doubly committed Th2+1 cells in effective clearance of SARS-CoV2 infections and contributes to our understanding that CD4+ T cells exist on a continuum retaining the capability to flexibly reprogram cytokine expression.

Camryn Foster

Optimizing Longitudinal Peripheral Blood–Based Immune Monitoring in Cancer Patients by Single-Cell Mass Cytometry

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Mass cytometry by time-of-flight (CyTOF) enables high-dimensional, quantitative profiling of millions of immune cells at single-cell resolution by multiplexing dozens of metal-tagged antibodies. This technology provides unprecedented resolution for deconvoluting the composition, abundance, and functional state of peripheral immune cells. However, the impact of longitudinal, peripheral blood–based deep immune profiling on the development of next-generation predictive and prognostic biomarkers for cancer patients remains elusive. In this study, we first assessed the resolution of the Maxpar immune profiling panel in several cohorts of cancer patients undergoing radiotherapy at our institution. Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood using Ficoll density gradients at baseline and at least one post-treatment time point. Deep immune profiling was performed

with the 30-marker Maxpar Direct Immune Profiling Assay (MDIPA), enabling detection of 37 immune cell subsets in approximately 0.5 million cells, and was expanded by six activation/exhaustion markers (PD-1, TIGIT, CD69, LAG-3, TIM-3, NKG2A) to further characterize immune cell functional states. Data were initially processed with the automated Maxpar Pathsetter software, which identifies 37 immune populations but provides limited support for in-depth exploratory analysis. To extend these analyses and integrate the additional functional markers, we applied a Pathsetter-derived gating strategy in Cytolution, a cloud-based platform for high-dimensional CyTOF data analysis, enabling validation and refinement of Pathsetter results as well as dimensionality reduction (UMAP), clustering, and cell-type annotation. Well-characterized clinical trial cohorts served as a training set to iteratively expand

and optimize the panel, which is now being applied to a large retrospective cohort of matched baseline and post-RT samples. These efforts aim to enhance the identification and functional characterization of relevant immune subpopulations and to guide the development of next-generation immune profiling tools to individualize therapeutic strategies for cancer patients, particularly those undergoing high-precision radiotherapy.

Bastian Hartmann

Spatially resolving the cell-matrix architecture by Imaging Mass Cytometry

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Spatial mapping of the cell landscape in relation to the extracellular matrix (ECM) architecture has not been investigated in detail. Their co-localization is essential to comprehend the complexity of the tumor microenvironment (TME) potentially identifying critical TME relations impacting patient survival and therapy response. To decode the dynamic interplay between cell-ECM interactions, we developed a specialized Imaging Mass Cytometry (IMC)-based antibody panel targeting key cell types in combination with ECM components, facilitating comprehensive characterization of the TME. Our novel ECM antibody panel captures interstitial and basement membrane ECM subtypes, as well as localizing a subset of secreted molecules. Initially, we performed pseudo-cell analysis of the ECM sections of the whole tissue along with real cell segmentation using key cell markers. These segmentations were combined to generate an approximation map of co-localization

Tran Luong

Immune phenotyping of canine peripheral leukocytes by mass cytometry

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Dogs are a key non-rodent species in preclinical safety studies, especially in pharmaceuticals, due to their close physiological, metabolic, and immunological resemblance to humans. Consequently, immunophenotyping canine peripheral blood mononuclear cells (PBMCs) is vital for translational research, immune monitoring, and safety assessments in drug development. However, the limited availability of canine-specific antibodies restricts detailed and accurate immune profiling, which is essential for advancing safety evaluations in drug development.

To address this challenge, we developed a 15-marker panel for comprehensive mass cytometry-based immunophenotyping of cryopreserved canine PBMCs. This panel covers major leukocyte subsets, including B cells, CD4+ T helper cells, regulatory T cells, CD8+ cytotoxic T cells, memory T cell subsets, natural killer T cells, natural killer cells, dendritic cells, CD4+ monocytes, classical monocytes, and neutrophils. The panel was rigorously optimized and validated on both the CyTOF XT® and Lunarion® mass cytometry platforms, demonstrating consistent performance across instruments. Importantly, the implementation of mass cytometry allows the generation of a backbone panel, to which additional markers can be added without extensive panel design or modification of the backbone itself.

The poster presents the full gating strategy applied on both CyTOF XT® and Lunarion® instrument and provides a comparative analysis of leukocyte subset frequencies as assessed by both mass cytometry platforms. of secreted soluble ECM molecules with structural ECM components and cell types. We further

applied a fiber segmentation approach designed to accurately delineate fibrillar ECM structures within IMC datasets. This method enables detailed investigation of co-localization patterns between the structural ECM and soluble ECM molecules, such as chemokines, which are crucial for understanding immune cell recruitment and signaling within tumors. This approach allowed us to visualize and analyze the co-localization of netrin-1, collagens, and chemokines in a fresh-frozen clear cell renal cell carcinoma sample thereby determining the relation of ECM-anchored netrin-1/chemokine complexes with the TME. Using this unique IMC panel, unknown ECM protein complexes and their cell association have been identified.

Ke Meng

Profiling the immune landscape of juvenile myelomonocytic leukaemia (JMML) reveals targets for checkpoint therapy

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Background: Juvenile myelomonocytic leukemia(JMML) is characterized by constitutive activation of the RAS signaling pathway with recurrent mutations in PTPN11, NRAS, KRAS, NF1, and CBL. Allogeneic hematopoietic stem cell transplantation (HSCT) is the only curative treatment option. However, Patients with PTPN11 mutations always have a high relapse rate after HSCT. The mechanisms underlying the poor clinical outcomes are unknown but likely due to differential oncogenic signaling connected to immune escape.

Method: We performed cytometry time-of-flight (CyTOF) analysis for a comprehensive human immune profiling to characterize the immune distribution and immune checkpoints of paired bone marrow and peripheral blood (n=11) from patients and healthy PBMC controls(n=4).

Results: Several immune clusters were identified including NK cells, T cells, stem cells, B cells, pDC-like cells and myeloid cells in bone marrow and peripheral blood. T-SNE plots showed that JMML cells highly expressed Tim-3, CD39, Siglec7, Siglec9, CD47, CD38 and NOX2; while the expression of PD-1 and CTLA-4 was observed in some CD8+T cells. JMMLPTPN11-mutant relapse group exhibited a notable increase in stem cells and monocytes. Furthermore, JMML PTPN11-mutant cells from the relapse group had a higher expression level of CD73 compared to those from the non-relapse group, but this difference was observed only in peripheral blood and not in bone marrow.

Conclusions: CyTOF analysis could identify different immune cell populations and immune signatures associated with JMML PTPN11 mutation. This information might provide potential immune targets for developing novel therapeutic approaches combining oncogenic signal inhibition and immunotherapy.

Julia Nguyen

Profiling protease activity in acute lymphoblastic leukemia with chemical probes

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Acute lymphoblastic leukemia (ALL) is the most common pediatric malignancy, originating in the bone marrow the primary site of blood cell production and maturation. In ALL, malignant cells replace healthy hematopoietic precursors, impairing marrow function and disrupting hematopoiesis. Diagnosis

relies on bone marrow aspiration supported by immunological and cytogenetic profiling. Despite cure rates exceeding 90%, about 10% of pediatric patients remain resistant to standard therapies. In this project, we investigate mechanisms of treatment resistance by analyzing proteolytic enzymes implicated in leukemia progression, including MALT1, cathepsins S, B, L, legumain, ADAM10, and the 20S proteasome. Conventional fluorescent activity probes assess only single enzymes and lack the capacity for multiplexed single-cell analysis. To overcome this limitation, we develop lanthanide-labeled probes based on selective peptide inhibitors of individual proteases. These peptide sequences form the basis of a theranostic platform: they can function either as diagnostic probes tagged with stable metal isotopes for enzyme activity profiling, or be converted into prodrugs specifically activated by the same proteases. Mass cytometry is an advanced single-cell technique widely used in immunology and oncology to analyze over 50 biomarkers simultaneously via distinct lanthanide isotopes, enabling detailed characterization of cellular subsets, functional states, and protein expression. However, conventional approaches are limited to measuring protein levels and cannot directly report enzyme activity. Currently, these probes are being tested in leukapheresis samples for optimization, with the aim of future application in bone marrow. By integrating lanthanide-tagged probes with mass cytometry, we enable multiplexed monitoring of proteolytic enzyme activities in individual leukemia cells.

Emily Riemer

Characterisation of the tumour immune microenvironment in bladder cancer: Implications for Predicting and Improving Therapeutic Response

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The tumour immune microenvironment (TIME) in solid tumours can be categorised into distinct states, including an "immune hot environment", which is characterised by high T cell infiltration; an "immune desert environment", which has few/no infiltrating T cells; an "inflamed, infiltrated but suppressive environment"; and an "immune cell excluded" environment.

To date, the TIME in bladder cancer has primarily been studied using transcriptomic deconvolution or single-cell RNA sequencing. However, spatially resolved analyses are currently lacking.

In this study, we are performing imaging mass cytometry using the Human Immuno-Oncology Panel and the Human Immune Cell Expansion Panel (Fluidigm) on 60 samples from 35 patients with bladder cancer.

Here, we present initial results from three patients. A195B displayed high infiltration of CD8+/PD-1+ T cells in tumour and stromal areas, whereas X055B and X057B harboured lower numbers of CD8+ T cells. A high proportion of CD68+ and CD206+ macrophages were found in samples A195B and X057B and were specifically localised at the tumour/stroma interface in A195B. In these areas, PD-L1 was also detected.

To associate TIME features with therapy, we measured activation-induced markers on T cells following treatment with nivolumab in patient-derived fragment cultures. Despite the presence of CD8+/PD-1+ cells, we did not observe activation of CD8+ T cells in A195B, indicating that these alone do not always predict functional immune responses. This highlights the importance of considering their spatial localisation and presence of immunomodulatory cell subsets.

Together, our initial results highlight the potential of combining IMC with preclinical testing of therapeutic responses to define how the TIME is associated with clinically relevant responses to therapy.

Felix Roettele

Insights into immune pathogenesis and response to therapy of Crohn's disease patients by imaging mass cytometry

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Background & aims: Advanced therapies such as TNF-antibodies (e.g. adalimumab, [ADA]) or the IL-12/23 antibody ustekinumab (UST) often fail to induce durable remission in Crohn's disease (CD). Previous studies suggest that exhausted CD8+ T-cells are associated with milder disease, whereas frequencies of IL 17A–producing CD8+ T-cells are increased during active inflammation1-3.

Methods: Ileal biopsies from 86 CD patients were included. 54 received therapies with ADA or UST (n=27, each), while 32 patients did not receive advanced therapy. A 42 plex imaging mass cytometry (IMC) panel was used to delineate spatial relationships and macro anatomical niches of tissue resident, potentially exhausted (PD 1[+]), and IL 17A[+] CD8+ T cell subsets, and to characterize their interactions with myeloid and stromal compartments.

Results: PD 1[+] CD8+ T-cells showed no clear association with disease activity but were strongly enriched within lymphoid follicles. Tissue resident CD8+ T-cells, mainly located in the epithelium, were enriched in remission as well as in chronic disease. IL 17A[+] CD8+ T-cells correlated positively with disease progression and formed their own niche. TREM1^{+} myeloid cells exhibited significant spatial proximity to all three mentioned CD8+ T cell subsets, implicating them in orchestration of CD8+ T cell phenotypes, while PDPN[+] fibroblasts emerged as key stromal contributors to active inflammation.

Conclusions: Spatially resolved profiling reveals a shift in CD8+ T cell phenotypes along the disease course of CD — from tissue resident to IL 17A– producing effector states— potentially coordinated by TREM1^{+} myeloid cells and PDPN[+] fibroblasts. These microenvironmental interactions highlight candidate prognostic biomarkers and therapeutic targets in CD.

Ann-Christine Severmann

The epigenetic landscape of osteoarthritis: Investigating histone modifications in primary human articular chondrocytes

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Osteoarthritis (OA) affects 500.000 patients worldwide, yet its molecular mechanisms remain poorly understood. Transcriptional and protein-level changes are likely linked by epigenetic marks, especially histone modifications. We aim to map the epigenetic profile of human articular chondrocytes (hACs) from OA patients and non-OA controls at single cell resolution using CyTOF. However, no existing protocol supports the analysis of freshly isolated hACs, capturing dynamic post-translational modifications like histone marks. We developed a procedure tailored to the low abundance and adhesive properties of hACs.

For CyTOF, hACs were stained using metal isotope-labelled antibodies, DNA was detected by Iridium and chondrocyte identity and quality were assessed via CD44 and SOX9. Preliminary optimisations were performed by flow cytometry.

Freezing of freshly isolated hACs resulted in poor cell recovery, promoting a switch to storage of pre-fixed cells. Protein-free buffers increased adhesion and were replaced by protein-containing options. Multiple permeabilization regimes were tested; Treatment with 1% SDS and 0.1% Triton X-100 for 10 min effectively disrupted the pericellular matrix, permeabilised membranes, and achieved chromatin accessibility. Combining permeabilization and blocking minimized cell loss. BSA-coated and low-retention tips increased cell loss, while FBS-coated tubes improved retention.

Our optimised protocol provides sufficient hAC recovery and allows detection of chondrocyte markers and histones modifications on single-cell level. Next, we will analyse patient samples to discover epigenetic modifications promoting OA and locate the disease relevant chondrocytes using

imaging mass cytometry.

Luka Tandaric

Peripheral blood leukocyte signatures as biomarkers in relapsed ovarian cancer patients receiving combined anti-CD73/anti-PD-L1 immunotherapy in Arm A of the NSGO-OV-UMB1/ENGOT-OV30 trial

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Immune checkpoint inhibitors have demonstrated limited efficacy in overcoming immunosuppression in patients with epithelial ovarian cancer (EOC). Although certain patients experience long-term treatment benefit, reliable biomarkers for responder pre-selection and the distinction of dominant immunosuppressive mechanisms have yet to be identified. Here, we used a 40-marker suspension mass cytometry panel to comprehensively phenotype peripheral blood leukocytes sampled over time from patients with relapsed EOC who underwent combination oleclumab (anti-CD73) and durvalumab (anti-PD-L1) immunotherapy in the NSGO-OV-UMB1/ENGOT-OV30 trial. We found survival duration was impacted by baseline abundances of total peripheral blood mononuclear cells. Longitudinal analyses revealed a significant increase in CD14+CD16- myeloid cells during treatment, with significant expansion of monocytic myeloid-derived suppressor cells occurring in patients with shorter progression-free survival, who additionally showed a continuous decrease in central memory T-cell abundances. All patients demonstrated significant PD-L1 upregulation over time on most T-cell subsets. Higher CD73 and IDO1 expression on certain leukocytes at baseline significantly positively correlated with longer progression-free survival. Overall, our study proposes potential biomarkers for EOC immunotherapy personalization and response monitoring, however, further validation in larger studies is needed.

Lei Zhu

Advancing the Characterization of Circulating Tumor Cells with High-Dimensional Mass Cytometry

Lei Zhu[1], Julia Cremer[2], Christiane Rutenberg[2], Amir Abdollahi[2], Sebastian Schölch[1] and Aoife Ward Gahlawat[2]

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Circulating tumor cells (CTCs) are rare cancer cells released from primary or metastatic sites into the bloodstream, and their detection holds great potential for diagnosis, prognosis and therapy monitoring. However, reliable methods for CTC isolation and characterization remain limited. This study explores the application of mass cytometry for CTC detection and characterization, leveraging its unique strengths in high-dimensional single-cell analysis. Cytometry by time of flight (CyTOF) offers high resolution, high sensitivity and the ability to simultaneously quantify more than 40 parameters without spectral

overlap, making it ideally suited for detecting rare cell populations such as CTCs.

To enable robust identification of CTCs, an antibody panel was established by extending the standard Maxpar® DirectTM Immune Profiling Assay (MDIPA) framework with cancer- and proliferation-associated markers, including Ki-67, EpCAM, and pan-cytokeratin. Building on this panel design, antibody titration experiments were performed for selected markers to determine optimal staining concentrations.

CyTOF-based detection was compared with the DEPArray system, an independent single-cell technology that enables imaging and physical isolation of single cells. While DEPArray allows precise recovery of single, viable or fixed cells for downstream analysis, it is limited in throughput and panel complexity. In contrast, CyTOF provides high-content, high-throughput, population-level analysis, although it does not enable downstream analysis of individual cells.

Together, these data demonstrate that CyTOF represents a powerful and scalable platform for multiparametric CTC profiling, paving the way for clinical applications in precision oncology.

Jennifer Zimmermann

Ex-vivo Modeling of Metabolic Niches to explore Tumor-Immune Interactions in CRC patient-derived Organoids

Jennifer Zimmermann[1], Lena-Marie Schmit[t1], Simon Frank[1], Aoife Gahlawat[2] , Luca Brenker[3], Dominik Niopek[3], Felix Hartmann[1,4]

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Metabolic heterogeneity within the tumor microenvironment profoundly shapes immune responses and contributes to disease progression, yet ex-vivo model systems to study these interactions in a human context remain limited. Here, we establish a patient-derived colorectal cancer organoid platform to dissect the role of metabolic niches in regulating immune cell function. Using CRISPR/Cas9 technology, we introduce targeted knockouts in key metabolic pathways to generate organoids with distinct metabolic phenotypes.

These organoids are co-cultured with macrophages, central regulators of inflammation and tissue remodeling, whose phenotype and function are tightly regulated by metabolic cues within the TME. In colorectal cancer, macrophages can polarize toward pro-inflammatory, glycolysis-dependent M1-like states that support anti-tumor immunity or toward oxidative phosphorylation− and fatty acid oxidation−dependent M2-like states associated with immunosuppression and tumor progression, which is highly influenced by nutrient competition and metabolite availability in the TME. Our model enables the systematic investigation of how tumor-intrinsic metabolic alterations modulate macrophage activation states, metabolic reprogramming, and effector functions. This metabolic niche model system provides a controlled ex-vivo framework to explore the crosstalk between cancer cell metabolism and the immune compartment, with the potential to reveal novel mechanisms of immune regulation and therapeutic vulnerabilities.

Tuesday, 27.01.2026

Product Feature Talks by Standard BioTools

Chairs: Aoife Gahlawat, Heidelberg

Cheuk Ting Wu

Institute of Anatomy, University of Bern

Imaging Mass Cytometry as a powerful platform to map dynamic liver repair after spatially fractionated radiotherapy (SFRT)

Radiation induced liver toxicity remains a major obstacle preventing radiotherapy from becoming a standard treatment for primary liver cancer, particularly hepatocellular carcinoma, which is frequently resistant to systemic therapies. Spatially fractionated radiotherapy (SFRT), including microbeam and minibeam, has emerged as a promising treatment in several preclinical cancer models by delivering very high peak doses through narrow beamlets while sparing the surrounding normal tissues (Engels et al. 2025; Fernandez-Palomo et al. 2020; Smyth et al. 2016; Trappetti et al. 2021, 2025). The recent successful clinical implementation of minibeam radiotherapy at the Mayo Clinic (Grams et al. 2024) further highlights its translational potential.

Despite encouraging outcomes, SFRT has never been studied in the liver due to the extreme radiosensitivity in the liver, and the underlying biological mechanisms driving normal tissue sparing and tumour control remain poorly understood.

In this project, we aim to evaluate SFRT as a therapeutic strategy for hepatocellular carcinoma by characterizing both normal tissue responses and antitumour efficacy. Using Imaging Mass Cytometry with a liver tailored antibody panel, we can resolve dynamic spatial interactions between hepatic and immune cell population in normal liver following microbeam irradiation. In the coming phase, we will extend this analysis to irradiated tumour models to identify cell type specific responses and mechanistic pathways that may contribute to therapeutic benefit.

Jayden Gittens

University of Liverpool, UK

Characterising the tumour microenvironment of liver metastases with imaging mass cytometry

Liver metastasis is a major driver of cancer-related mortality worldwide. Colorectal cancer (CRC) has a high propensity to metastasise to the liver, engendering poorer outcomes for patients. To investigate the microenvironmental features that define the metastatic site, we employed Hyperion IMC.

We leveraged a TMA of 25 matched primary and metastatic CRC patients. Our 21-plex antibody panel targeted major cellular lineages found within the TME. The IMCSegmentationPipeline was used to segment and pre-process images for downstream phenotyping and spatial analyses. 13 cell types identified by IMC. Myeloid and stromal cells were the most abundant within the TME. Clustering revealed enrichment of two TME cellular neighbourhoods

(CNs) at the metastatic site, where CN 2 comprised primarily macrophages and stromal cells. Further spatial analysis revealed significant pairwise interactions between M2 macrophages and Collagen 1+ fibroblasts, Vimentin+ fibroblasts, and neutrophils.

With Hyperion IMC we are deconvoluting the spatial architecture found in liver metastases. We also aim to further characterise the TME with GeoMx to complement our findings on a transcriptional level.

9:30 am - 10:45 am Suspension MC Session

Chairs: Sabine Baumgart, Jena & Sarah Warth, Ulm

Invited Talk by Olivier Molendi-Coste

University of Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, US 41 - UAR 2014 - PLBS, F-59000 Lille, France

Batch Effects Correction in Mass Cytometry Data

High-content cytometry allows a fine characterization of cell populations in a single sample thanks to the analysis of several dozen targets in a unique panel. Experimental solutions and detailed data processing pipelines were developed to reduce both the staining conditions variability between samples and the number of tubes to handle. This refinement and the possibility of multiplexing several samples (barcoding) have interested large-scale studies. However, an unavoidable variability appears between samples, barcodes, series and instruments (in multicenter studies) contributing to "batch effects" that must be properly controlled.

To correct these undesirable effects, several approaches (clustered or not) and methods (linear or quantile segmented) have been proposed, based or not on a reference sample present in each batch, but they all lack transpacency, intuition and user-friendlyness.

We created a dedicated package named CytoBatchNorm, based on the CytofBatchAdjust script by Schuyler et al., which uses the unclustered events of the reference sample to calculate linear corrections. It provides a graphical interface allowing users to define a specific correction for each marker in a single run, with graphs visualization that guides users through quickly setting the parameters. It allows corrections to be previewed and inter-marker effects to be checked as the settings are updated. CytoBatchNorm will help the cytometry community to adequately scale data between batches, reliably reducing batch effects and improving subsequent dimension reduction and clustering.

Short Talk by Mehmet Serdar Koca

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

Center Harmonization for Multicenter CyTOF Immune Monitoring

3TR is a multicenter consortium aiming to identify shared molecular signatures across seven chronic immune-mediated diseases. As part of this effort, immunophenotyping data will be generated for more than 2,500 samples, requiring a harmonized and optimized multicenter CyTOF acquisition strategy.

Optimize the sample acquisition protocol for multicenter CyTOF studies and determine whether data acquired across different instruments can be aligned.

A pilot experiment was performed to optimize acquisition using 46-plex panel together with barcoded whole-blood samples from three donors and compensation beads. The samples were prepared, frozen, and later acquired on one three HELIOS, and one CYTOF XT instrument across three acquisition batches. Sample acquisition scheme varied by batch: Batch 1 used one long run per aliquot, while Batches 2−3 used two shorts runs. PCA was applied using median signal intensities (MSI) to evaluate the effect of acquisition time and aliquot used within and across centers. Main cell populations were manually gated and MSI, coefficients of variations (CV) were calculated. Center enrichment and batch effects were assessed.

The compensation matrix generated using the brightest cytometer (Center1), demonstrated the most effective spillover correction. Flow rate instability contributed to signal drifts in several markers, affecting background-related intensities and cell-associated signals The PCA analysis showed that restricting the analysis to the first 65 minutes of acquisition improved clustering for all centers. Bead-

based normalization reduced center-associated variability, resulting in CV values of MSI and cell frequencies below 20% for most cell populations. Samples clustered according to the donors, but center effect was observed, thus requiring reference-based normalization.

Multicenter CyTOF experiments are feasible, however special sample acquisition protocol and additional reference-based normalization are required to align the data.

Short talk by Lucía Rodríguez Doña

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

What it takes to CyTOF-profile 2,500 clinical samples across four immune-mediated diseases

Background: 3TR is a multicenter consortium studying immune-mediated diseases to find common molecular signatures. Mass cytometry is employed to profile immune cell populations.

Objective: Establish a standardized staining and antibody-validation protocol to enable immune monitoring of >2,500 blood samples from 3TR clinical studies.

Methods: To ensure consistent recruitment, whole blood was fixed and stored at -80°C until staining. Antibody panel development included clone selection in fresh blood, antigen stability testing and titration in fixed/frozen samples from healthy donors and patients, using flow or mass cytometry. Large-scale metal conjugation was performed for higher yields. To improve technical robustness, two protocols were tested. Cells from 3 donors were thawed, lysed, aliquoted and either refrozen immediately or barcoded, pooled and refrozen at -80°C. Depending on the workflow, samples were subsequently

barcoded and pooled or immediately stained with the frozen antibody cocktail.

Results: Of 12 antibody clones evaluated by flow cytometry, 8 met criteria for inclusion. Overall, 55 markers were titrated in CyTOF and 50 approved; 35 antibodies were conjugated in-house, 21 at large scale. The final panel includes 42 common and 4 disease-specific markers, comprising lineage and functional markers. Panel was tested following the described workflow. Several markers displayed shifts in signal intensity between 1-week and 1-month storage. Consequently, a maximum 10-day storage period before staining was established.

Conclusions: Standardized CyTOF protocol and 46-plex antibody panel enable robust and reproducible immune profiling of over 2,500 samples from patients, supporting biomarker discovery and personalized therapies.

Short Talk by Niclas Schierloh

Department of Internal Medicine II, Medical Center - University of Freiburg, Freiburg, Germany

SceniTOF - Functional single-cell multiplexed metabolic profiling to map bioenergetics of heteroegeneous immune cells by mass cytometry in mice and men

Immune cell activation, differentiation, and effector function are tightly coupled to cellular metabolism, but many functional metabolic assays lack single-cell resolution. Single-cell metabolic regulome profiling (scMEP) by mass cytometry quantifies metabolic proteins alongside immunophenotypes, but protein levels do not necessarily reflect metabolic activity or flux. The method SCENITH (Single Cell ENergetIc metabolism by profilIng Translation inHibition), developed by flow cytometry, may overcome this limitation by quantifying pathway-specific effects

on protein synthesis after brief ex vivo inhibitor treatment.

Here we established a mass cytometry-compatible version of the SCENITH assay using a secondary metal-coupled antibody to detect the translation inhibitor puromycin as an integral part of the SCENITH assay and combine it with the scMEP approach. Briefly, human or murine immune cells are treated ex vivo or after *in vitro* stimulation with inhibitors of glycolysis and oxidative

phosphorylation, pulsed with puromycin, barcoded, and stained with a multiplexed CyTOF panel targeting metabolic regulators, lineage markers, and activation states. This combined SCENITH−CyTOF approach enables scalable mapping of energetic pathway dependence while concurrently resolving phenotypic and metabolic profiles. We demonstrate the applicability of the SceniTOF assay to identify the differences in metabolic state and flux across human T cell differentiation states and the murine B cell compartment.

Together, this integrated approach links multiplexed single-cell phenotyping with functional metabolic readouts, allowing SceniTOF to relate metabolic potential to actual metabolic flux by mass cytometry.

10:45 am – 11:30 am   *Coffee break*

11:30 am - 1:15 pm   News from… Part II

Chairs: Claudia Peitzsch, Dresden & Lena Müller, Vienna

News from Ulm: Dominik Schlotter

Department of Obstetrics and Gynecology, Ulm University, 89075 Ulm, Germany

Multiparametric mass cytometry (CyTOF) for exploring the sex-dependent crosstalk between DNA damage and inflammatory response during aging

The widely acknowledged sexual dimorphism in life expectancy (4 years), immunosenescence, and the incidences of age-related diseases provide evidence for sex differences in the aging process. Previously, we showed that peripheral blood lymphocytes (PBL) from older men and women differentially regulate multiple DNA damage and replication stress response pathways differentially contributing to the increase in genomic instability with age. Genomic instability and replication stress can lead to the release of DNA fragments from the nucleus into the cytoplasm, where these fragments activate the cyclic GMP-AMP synthase (cGAS) causing downstream activation of the stimulator of interferon genes (STING). Subsequent signaling involves NF-κB activation, senescence, and inflammation leading to immune cell recruitment. To correlate the crosstalk between replication stress, DNA damage response (DDR), and inflammation and to identify sex-differences within, we use multiparametric mass cytometry (CyTOF) as key method. To identify the immune cell subpopulations in cycling human PBL, we applied the Maxpar Direct Immune Profiling Assay (MDIPA) and added multiple markers for DDR, senescence, and cytoplasmatic DNA sensing. Ultimately, our panel consists of 47 markers including platinum-labeled antibodies. We successfully applied our panel to cycling human PBL after 72 h PHA-stimulating culture prior to staining. In total, we measured 85 samples from young (<26) and older (>60) men and women and have overcome obstacles caused by the experimental conditions required for DDR studies. Our ongoing data analysis shall reveal sex differences in the age-related amplification of DNA damage and inflammation.

News from Berlin (DRFZ): Axel Schulz

German Rheumatology Research Center (DRFZ), a Leibniz Institute, Berlin, Germany

Comparative Performance Benchmarking of Lunarion vs. Helios and CyTOF XT Mass Cytometers

The Lunarion mass cytometer (Polaris Biology) is a recently developed platform for high-throughput, high-dimensional single-cell analysis. Installed at LIH (Luxembourg) and DRFZ (Berlin), its performance was evaluated in direct comparison with established Helios and CyTOF XT systems (Standard BioTools). Benchmarking was performed using standardized multi-element solutions, metal-labeled beads, and complex biological samples to assess the instruments under realistic operational conditions.

Sensitivity profiling using multi-element solutions

revealed highly consistent performance across all channels, with near-identical signal curves between the two Lunarion instruments and approximately fivefold higher sensitivity relative to the CyTOF XT. Analysis of 46 single-stained, metal-labeled compensation beads spanning the mass range from ^{89}Y to ^{209}Bi demonstrated an average one-log increase in signal intensity across most channels on the Lunarion compared with the Helios system. This gain was particularly pronounced in the lower atomic mass range (<150 amu). Importantly, it affected both positive and negative bead populations and was associated with a slightly higher staining index in most, though not all, channels. Coefficients of variation (CVs) were similar to those observed on the Helios and typically remained below 30%, indicating comparable measurement precision.

To evaluate performance on biological samples, phospho-signaling responses were analyzed in human whole blood from two donors following stimulation with innate immune ligands at multiple time points, which were barcoded using a combined beta-2-microglobulin and TeMal-based approach. Both platforms reliably delineated major immune populations and retained donor-specific phosphorylation profiles. Signal kinetics and fold-change dynamics for key markers such as p-p38 and pTBK1 (e.g., in classical monocytes) were closely aligned across instruments, supporting cross-platform reproducibility of cellular response measurements.

News from Luxembourg: Antonio Cosma

National Cytometry Platform, Luxembourg Institute of Health, Luxembourg

Democratization of mass cytometry and data analysis (#BI4Flow)

The recent deployment of the Lunarion benchtop mass cytometer represents a transformative shift toward the democratization of mass cytometry in research and clinical settings. Historically, mass cytometry has been confined to specialized core facilities requiring dedicated operators and substantial infrastructure, limiting accessibility for individual research groups. The Lunarion's user-friendly design and simplified maintenance protocols enable scientists to operate the instrument directly, effectively transferring analytical capacity from core facility specialists to end-users. This democratization parallels the paradigm shift initiated by Business Intelligence (BI) tools in the corporate analytics landscape, where domain experts gain direct access to sophisticated analytical capabilities without requiring specialized technical expertise. By integrating Lunarion's operational accessibility with BI principles—including interactive data visualization, intuitive interfaces, and seamless data sharing mechanisms—we can establish a framework that empowers immunologists and cell biologists to conduct complex multidimensional analyses independently. This integration not only accelerates scientific discovery through rapid iteration and hypothesis testing but also fosters collaborative science through interactive visualizations that enable non-expert stakeholders to explore and interpret high-dimensional single-cell data. We propose that this convergence of accessible hardware, user-centric software design, and business intelligence methodologies will fundamentally reshape how single-cell analysis is conducted across academic, clinical, and industrial research environments.

News from Halle: Marcus Bauer

Institute of Pathology, University Medicine Halle, Martin Luther University Halle-Wittenberg, 06112 Halle (Saale), Germany

Section of Immunopathology, Institute of Pathology, Martin Luther University Halle-Wittenberg, 06112 Halle (Saale), Germany

Establishment of Imaging Mass Cytometry for High-Dimensional Spatial Profiling of Decalcified Bone Marrow Biopsies

Background: Comprehensive spatial characterization of the bone marrow microenvironment in myeloproliferative neoplasms (MPN) is technically challenging due to tissue complexity and bone decalcification. Imaging mass cytometry (IMC) enables high-dimensional, spatially resolved

single-cell analysis; however, its implementation in decalcified bone marrow biopsies remains limited.

Methods: We established an IMC workflow for decalcified bone marrow biopsies using a panel of 32 metal-conjugated antibodies targeting immune, stromal, and proliferative markers. IMC was performed on 22 biopsies from MPN patients before treatment and after therapy with the JAK inhibitors Ruxolitinib or Fedratinib. Following image acquisition, robust single-cell segmentation and unsupervised clustering were applied to define cell (sub) populations. Distribution of cell (sub)populations and marker expression profiles were compared across treatment conditions to demonstrate the applicability of the approach.

Results: IMC analysis of decalcified bone marrow biopsies (whole slide) enabled the detection of major immune and stromal cell populations and identified 11 distinct cellular clusters based on the marker expression. Spatially resolved analyses revealed cell type-specific expression patterns and treatment-

associated shifts in the cellular composition. Bone marrow samples from Ruxolitinib-treated patients showed increased CD68+CD163- macrophage and myofibroblast populations accompanied by reduced expression of (immune) signaling markers (NFkB, pERK, pSTAT3) and proliferation (Ki-67) markers. In contrast, bone marrow samples from patients treated with Fedratinib had a decreased CD14+ monocyte abundance and increased expression of Ki-67 and NFkB compared to biopsies prior to treatment.

Conclusions: Here, we established a high-dimensional, spatial single-cell profiling of decalcified bone marrow biopsies. The presented workflow enables detailed characterization of the bone marrow microenvironment and provides a basis for future studies investigating treatment-induced remodeling in MPN and other hematologic diseases.

News from Jena: Sabine Baumgart

Institute of Immunology, Core Facility Cytometry, Jena University Hospital, Friedrich-Schiller-University Jena, Jena, Germany

Introducing UriTOF: A Preservation Workflow for Urine CyTOF

Objective: Mass cytometry (CyTOF) allows high-dimensional single-cell analysis but typically requires immediate sample processing, which limits its use for fragile, low-cell clinical specimens such as urine.

Methods: We developed UriTOF, a preservation strategy that enables delayed processing by combining cisplatin viability staining with gentle fixation and cryopreservation. The protocol was validated using PBMCs and applied to urine samples from kidney transplant recipients.

Results: Cell integrity, surface marker expression, and viability discrimination were maintained across freeze−thaw cycles and low cell inputs. Urine immune profiles were comparable to standard CyTOF workflows and enabled identification of urinary CD8$^+$CD38$^+$ T cells as a marker of T cell−mediated rejection, validated across multiple orthogonal platforms.

Conclusion: UriTOF enables delayed and standardized CyTOF analysis of low-yield urine samples, supporting clinical and translational immune profiling and facilitating non-invasive biomarker discovery in kidney transplantation.

News from Salzburg: Markus Steiner & Stephan Drothler

Markus Steiner[1], Stephan Drothler[1], Anna Kolmhofer[1,2]3, Christian Scherhäufl[1], Jan Philip Höpner[1,3], Heinz Hofbauer[4], Johannes Schuiki[4], Giulia Pessina[1], Alexander Egle[1], Andreas Uhl[4], Roland Geisberger[1] and Nadja Zaborsky[1]

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4Dept. of Artificial Intelligence and Human Interfaces, Paris-Lodron-University Salzburg, AustriaInteractive Cell Gating and ultra-specific detection of RNA: New IMC tools for focused microenvironment analysis

Image Mass Cytometry (IMC) enables the study of complex biological environments such as tumor-immune landscapes, where the spatial context of expanding tumor and immune cell clones is of high relevance. Current workflows cluster cells by their expression to cellular patches or neighborhoods, but intuitive tools for selecting individual cells based on their spatial location are currently lacking. Moreover, visualizing individual clones by their unique CDR3 region, which necessitates the detection of ultra-specific RNA, is currently not supported by standard IMC workflows. To address these problems, we present two novel tools.

Spatialgater provides an interactive web interface for spatial gating of cells in IMC data. Users can visualize cells on zoomable tissue images and select specific cells by drawing polygon gates. Cell identifiers from selected cells can be exported as CSV files or written back as logical gate columns in colData() of the SpatialExperiment for downstream analysis. We provide the function as an R package available at https://github.com/Mark-Ste/spatialgater.

While simultaneous detection of mRNA (>300 bp) is possible, there is currently no technique for the detection of RNA with single-base pair precision, required for the study of subclonal tumor evolution and identification of clonal expansion in situ. Therefore, we introduce a protocol for combined microscopic and IMC staining on single slides, enabling the integration of single base pair-specific RNA information and spatial protein multiplex data.

We showcase the usability and benefit for microenvironment analysis of the gating tool andultra-specific RNA-protein co-detection in a leukemic setting.

News from Cologne: Daniel Bachurski

Department I of Internal Medicine, Medical Faculty of the University of Cologne, and University Hospital Cologne

From TeLEV to MULTI-TeLEV – Simultaneous profiling of EV uptake and recipient cell signaling from six EV sources

Extracellular vesicles (EVs) are potent modulators of tumor immune microenvironments. To study these interactions at single-cell resolution, we previously developed TeLEV (Tellurium-based labeling of extracellular vesicle proteomes), utilizing the metabolic incorporation of L-2-tellurienylalanine (TePhe) to create mass-tagged EVs. Using this approach, we identified the specific induction of an interleukin receptor triad (CD25, CD123, CD127) and downstream STAT5 signaling as a functional consequence of B-cell malignant EV uptake.

In this update, we present MULTI-TeLEV (Multiplexed isotopically enriched TeLEV), a significant methodological advancement designed to interrogate complex EV environments. By synthesizing TePhe variants with distinct enriched tellurium isotopes, MULTI-TeLEV enables simultaneous tracking of EVs from up to six sources within the same experimental setting.

Leveraging this capability, we provide new insights into the rules of EV entry. We demonstrate that EV uptake is highly competitive. When recipient cells are exposed to multiple EV sources simultaneously,

uptake is not stochastic; rather, we observe distinct uptake patterns where specific EV sources are preferentially internalized over others. MULTI-TeLEV thus enables the precise deconvolution of source-specific uptake hierarchies that are invisible in conventional single-source assays.

1:15 pm - 1:55 pm Invited talk by Marcin Poręba, Wrocław

Chairs: Hyun-Dong Chang & DRFZ Berlin, Bertram Bengsch, University Medical Center Freiburg

Department of Chemical Biology and Bioimaging, Faculty of Chemistry, Wroclaw University of Science and Technology, Wroclaw, Poland

The application of chemical probes for imaging protease and kinase activity in tumor cells by mass cytometry.

Enzyme probes are small molecules that report catalytic function rather than expression. Activity-based probes (ABPs) combine a recognition element with a reactive "warhead" that forms a covalent adduct with the active site, while substrate-based probes are cleaved to release a reporter. Fluorescent probes have powered this field for decades, but spectral overlap limits multiplexing in complex samples. Mass cytometry enables high-dimensional single-cell profiling, but it largely reports protein abundance rather than enzymatic function. Our work brings these chemical biology tools to the CyTOF community by adapting them to lanthanide metal tags for suspension mass cytometry and to Hyperion imaging on tissue sections. In our work, we deploy metal-tagged inhibitor-based probes (called TOF-probes), providing direct, covalent readouts of active enzymes at single-cell resolution. Using this approach, we map the activity of cysteine cathepsins B, S, and L, as well as legumain, to define matrix-remodeling niches in cell-line models and in dissociated patient tumors. For cell death, a caspase-3 activity probe identifies apoptotic subpopulations that are missed by expression-only panels and that track with therapy exposure and response. Finally, neutrophil serine protease probes reveal activated neutrophils within PBMC cells and tumor samples. Finally, to expand our probe repertoire, we have recently developed metal-tagged kinase probes that directly label these enzymes in their active state. Moreover, our toolset integrates with metal-tagged antibodies, allowing us to monitor protease and kinase activity at single-cell resolution across defined cell subsets. So far we demonstrate significant potential to map tumor ecosystems focusing on enzyme activities, and within current research we are applying this approach to inflammatory disorders and neurodegenerative diseases.

1:55 pm – 2:15 pm Farewell & Award Ceremony

2:15 pm – 3:00 Lunch to go

3:00 PM – 4:00 PM Inaugural Assembly of the Mass Cytometry working group (Arbeitskreis Massenzytometrie) under the umbrella of the German Society for Cytometry (DGfZ)

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