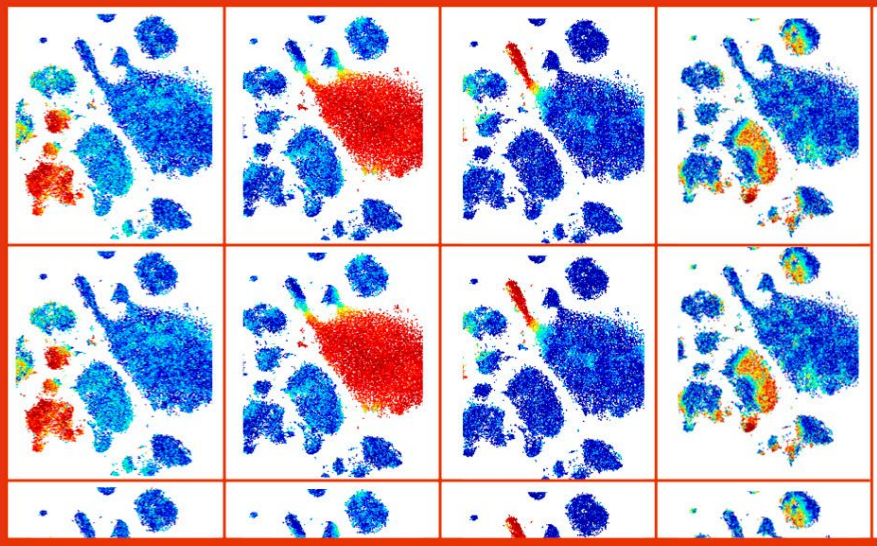
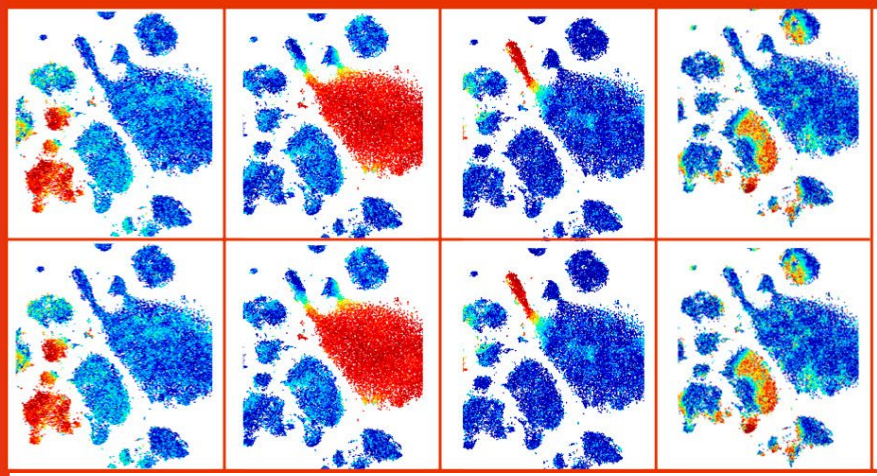


GerMaNet
German Mass Cytometry Network

# 3rd German Mass Cytometry User Forum



January 23-24, 2020
Berlin

# Abstract book

**Venue:**

KARL STORZ
Besucher- und Schulungszentrum Berlin
Scharnhorststraße 3
10115 Berlin Germany

*We are moste grateful to our Sponsors and Exhibitors*











*funded by*


*Dear friends and collegues,*

On behalf of the steering committee of the German Mass cytometry Network, I am pleased to welcome you to our 3rd German Mass Cytometry User Forum in Berlin. After very successful meetings in 2018 and 2019 I am looking forward to continue the series in 2020.

The organizing committee has put together an exciting 2-day program around different aspects of mass cytometry covering technology basics & reagents, computational data analysis, imaging mass cytometry and several applications in different fields such as immunology, oncology, and personalised medicine. Workshops on technology & reagents, and computational data analysis will provide the opportunity to ask questions and receive expert answers, and to discuss cutting-edge developments in the field. Plenty of time will be devoted to abstract presentations and poster discussions.

On Thursday evening, we invite you to discuss the posters, and to relax and mingle with your colleagues at the evening event generously sponsored by Fluidigm. I also would like to thank all commercial exhibitors for their generous financial support to make this meeting possible.

I am looking forward to an inspiring Mass Cytometry User Forum in Berlin and wish you all an exciting meeting and a pleasant stay in Berlin.

Yours,

Henrik Mei

# Thursday, January 23rd

9:00 Welcome (Henrk Mei)

## Session 1

Chairs: Bertram Bengsch & Henrik Mei

09:15 Antonio Cosma, Luxembourg: Business intelligence, number theory and mass cytometry

Henrik Mei, Berlin: Chronic inflammation

10:45 *Coffee break*

## Session 2 News from ...

Chairs: Bertram Bengsch & Henrik Mei

11:15 ... Freiburg: Bertram Bengsch - Understanding T cell exhaustion in different human disease entities

... Ulm: Fabian Gärtner - Phenotyping of immune cells in adipose and lean mice after thorax muscle trauma

Selected abstract:
Camila Fernandez-Zapata - Diversity of human myeloid compartment in active lesions of late stage multiple sclerosis determined by mass cytometry

12:45 *Lunch break*

## Session 3 - Presentation of selected abstracts

Chair: Axel Schulz

14:15 Selected abstracts:

Marie Urbicht - Dissecting immune cell modulation during therapeutic targeting of CD38 in Systemic Lupus Erythematosus using mass cytometry

Jessica Suwandi - 1 Multidimensional analyses of proinsulin peptide-specific regulatory T cells induced by tolerogenic dendritic cells

Marilena Letizia - Mass cytometry reveals effects of Store-operated Calcium Entry pathway in human intestinal inflammation

15:45 *Coffee break*

## Session 4 Workshop: Mass Cytometry Basics and Reagents

Chairs: Henrik Mei & Axel Schulz

16:30 ISAC Lecture:

Michael Leipold, Stanford: CyTOF in large studies: Challenges and lessons learned

Selected abstract:
Heidi Ødegaard Notø - Single cell gating by cell cycle analysis in mass cytometry

Pannel discussion: Moderator Axel Schulz

## Poster presentation and get together

Chairs: Désirée Kunkel & Sarah Warth

18:30-23:00 Poster Session and Dinner: generously supported by Fluidigm Corporation

# Friday, January 24th

## Session 5

Chair: Henrik Mei

9:00 Burkhard Becher, Zurich: High-dimensional cytometry for immunophenotyping

Thomas Höllt, Leiden: Explorative visual analytics for large single-cell data

10:30 *Coffee break*

## Session 6 Workshop Data Analysis

Chair: Marie Urbicht

11:15 Tyler Burns, Berlin: A visual interrogation of dimension reduction tools for single-cell analysis

Selected abstract:
Ina Stelzer - A third trimester multi-omic clock predicts the spontaneous onset of labor

Panel discussion Moderator Marie Urbicht, Berlin

13:15 *Lunch break*

## Session 7 News from...

Chair: Désirée Kunkel

14:45 ... Berlin (DRFZ): Andreas Grützkau - How many roads must a biomarker walk down, before you call it a BIOMARKER? Hope and Hype for new cellular biomarkers in rheumatology

... Berlin (BCRT): Thomas Sell - Modelling cell-type specific signalling networks by mass cytometry in intestinal organoids

Selected abstract:
Marieke Ijsselsteijn - Characterisation of granzyme b positive myeloid cells with immunotherapeutic potential for colorectal cancer patients using imaging mass cytometry.

16:15 *Coffee break*

## Session 8 Fluidigm Session

Chair: Henrik Mei

16:45 *Invited talk sponsored by Fluidigm:*

Marie-Laure Yaspo, Berlin: Systems omics for exploring melanoma subtypes

## Poster prize award and farewell

Chair: Henrik Mei

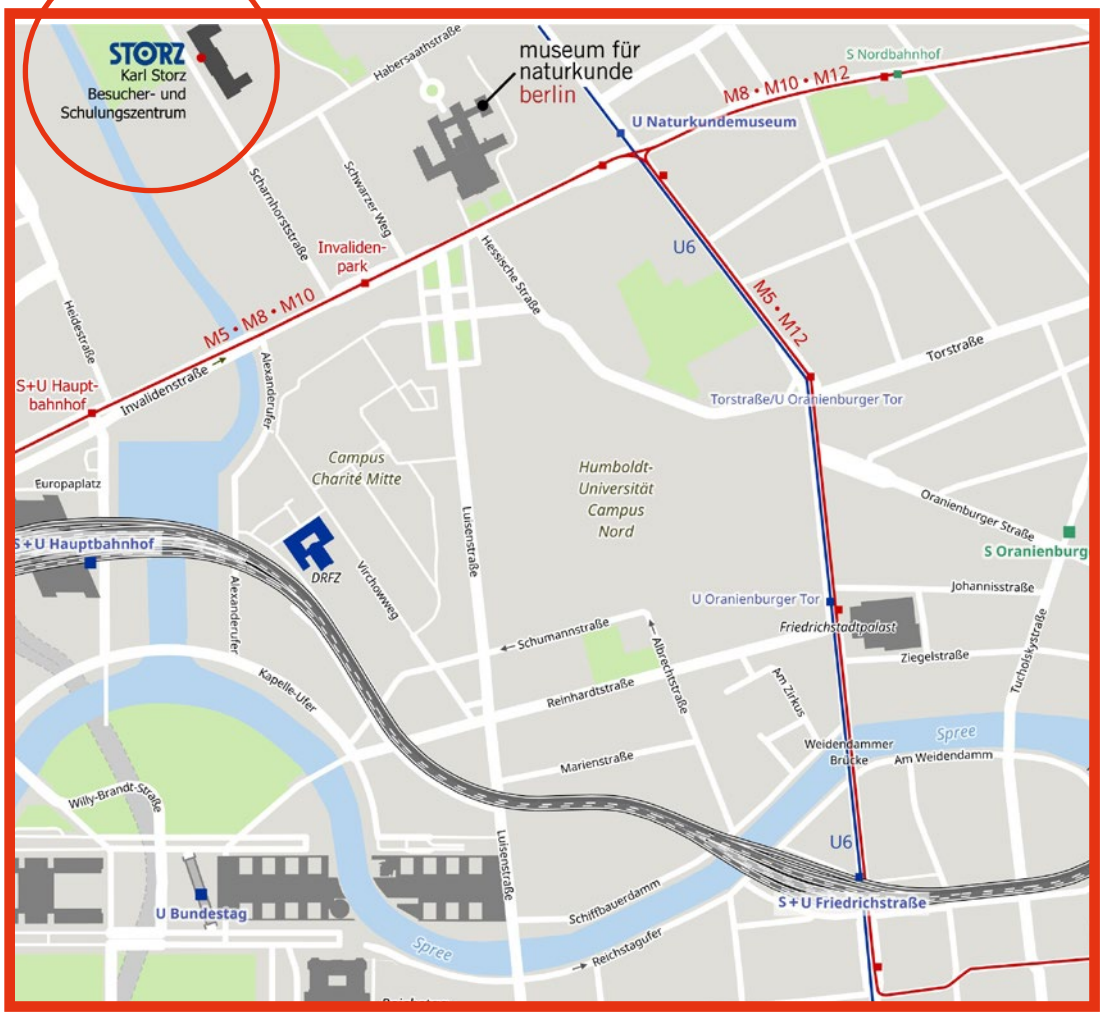
17:30 Poster prize (sponsored by Beckman Coulter Life Sciences) award ceremony

Wrap-up and farewell with soup

# Impressions of the 2nd German Mass Cytometry User Forum, 2019 in Berlin


# Venue of the 3rd German Mass Cytometry User Forum

**KARL STORZ**
**Besucher- und Schulungszentrum Berlin**
**Scharnhorststraße 3**
**10115 Berlin Germany**
**www.karlstorz.com**




**Distances**
Berlin-Hauptbahnhof
(Central Station): 1 Kilometer
Tegel Airport: 9 Kilometer
Schönefeld Airport: 25 Kilometer

Parking: No parking lots for guests

Public parking:
Car park Berlin-Hauptbahnhof, (Central Station)
Access routes: B96-Tunnel or Clara-Jaschke-Straße, 10557 Berlin
From there: Bus 120, Bus TXL, Tram M5, M8, M10 or approx. 12 min by foot

**Public Transport**
From Tegel Airport: TXL Express Bus to Invalidenpark Station

From Schönefeld Airport: Regional train RE7/ RB14 to Central Station (Hauptbahnhof), from there: see below - Hauptbahnhof

From Central Station (Hauptbahnhof):
Bus 120 to Scharnhorststraße/Habersaathstraße Station; from there: approx. 2 min by foot
or Tram M5, M8, M10/ Bus TXL
to Invalidenpark Station; from there: approx. 6 min by foot

# Thursday, January 23rd

## Session 1: Invited Talks I

Chairs: Bertram Bengsch & Henrik Mei

## Business intelligence, number theory and mass cytometry

**Antonio Cosma**

*Quantitative Biology Unit, LIH, Luxembourg*

Merging knowledge from different disciplines can open new perspectives and pave the way to unforeseen problem solutions. Here, I will present how "Business Intelligence" and "number theory" can help in our process to understand mass cytometry data.

Business Intelligence (BI) comprises the tools and technologies used to collect and analyze information in order to support the process of decision-making. Data management and data analysis are central to BI activities since they are the source of business decisions. Several BI solutions are available as open-source or proprietary software.

Number theory is a branch of mathematics dedicated to the study of natural numbers and tools used to manage them. In 300 B.C., Euclid set the basis of number theory with the "fundamental theorem of arithmetic" and, this day and age, number theory is at the basis of modern cryptography and internet security.

I will present some data scenarios in which mass cytometry data were analyzed using BI tools and some techniques to classify cell populations using number theory.

## Chronic inflammation

**Henrik Mei**

*Deutsches Rheuma-Forschungszentrum (DRFZ), Berlin, a Leibniz-Institute, Germany*

Chairs: Bertram Bengsch & Henrik Mei

## Understanding T cell exhaustion in different human disease entities

**Bertram Bengsch**

*University Medical Center Freiburg, Faculty of Medicine, Germany*

Exhausted T cells have been linked to unfavorable patient outcomes in cancer and viral infections but they are predicted to be beneficial in autoimmunity. We have recently established epigenomic and cytometric immune signatures of exhaustion in highly immunogenic cancers and HIV infection. We are currently using these novel signatures to identify the role of exhaustion in settings of autoimmune-mediated disease and lowly immunogenic cancers, such as Hepatocellular Carcinoma (HCC) using systems immunology approaches centered around high-dimensional mass cytometry profiling. In this presentation, we will discuss novel insights into the role of exhaustion obtained using these approaches.

## Phenotyping and gene profiling of immune cells in adipose and lean mice after thorax and muscle trauma

**Fabian Gärtner**

*Forschung Chirurgie 1 (AG Knippschild), Uniklinikum Ulm, Germany*

1. Introduction

The issue of obesity in our society grows bigger and bigger every year. Besides direct adverse effects on health and life quality of obese people, it can also affect the regeneration of the tissue after trauma. It has been shown in previous work, that obesity can influence the healing process after a traumatic injury in mice, that were suffering from a muscle trauma. This influence is amongst other things based on a dysregulation of macrophages and a resulting shift in the M1/M2 axis(1,2). We hypothesize that the immune cells have an increased inflammatory potential in obese animals and therefore lead to this shift towards a more pro-inflammatory environment. Therefore this work is focusing on polytraumatic injuries of the muscle as well as the lung and is phenotyping the immune cells after different time points after trauma.

2. Methods

The used mouse model for this study are female C57BL/6J mice, which are either lean or obese. The obesity is diet induced using a 60 % fat diet as induction. The polytrauma is induced using a drop tower for the muscle injury, followed by a lung injury induced with air pressure. Both methods set blunt traumas. The mice were kept for different time points and the blood, spleen and trauma tissue (muscle and lung) was harvested and analyzed by CyTOF, flow cytometry and RT-PCR, respectively.

3. Results

An increased pro-inflammatory response can be observed in obese animals in comparison to the lean counterparts. This can mainly be observed in the first 24 hours in the peripheral blood stream based on neutrophils and pro-inflammatory Ly6Chi monocytes, which are present in the periphery for a longer time in obese animals. Additionally the monocytic subsets of obese animals show an increased expression of the chemokine receptor CCR2, which is responsible for the recruitment of immune cells to the site of inflammation following a CCL2 gradient. These difference can also be observed at later time points in the gene profiles of the lung and the muscle. For example an increased ratio of M1 to M2 specific genes can be observed in obese individuals in the lung, whilst the regeneration process of the muscle is slowed down in obese animals based on the expression of Myog.

4. Discussion

These results indicate that the response of the immune system to the polytraumatic injury is delayed as well as prolonged. Additionally the increased expression of CCR2 on monocytes leads to an increased homing of the pro-inflammatory monocytes populations to the site of injury resulting in a shift of the M1/M2 axis and a delayed regeneration process.

## Diversity of human myeloid compartment in active lesions of late stage multiple sclerosis determined by mass cytometry

**Chotima Böttcher[1], M. Camila Fernández Zapata[1], Marlijn van der Poel[2], Stephan Schlickeiser[3], Cheng-Chih Hsiao[4], Mark R Mizee[2], Maria C.J. Vincenten[2], Désirée Kunkel[3], Inge Huntinga[2,5], Jörg Hamann[2,4], Josef Priller[1,6,7]**

*[1]Charité Universitäatsmedizin, Germany; [2]Neuroimmunology Research Group, Netherlands Institute for Neuroscience, Amsterdam, The Netherlands.; [3]Berlin-Brandenburg Center for Regenerative Therapies (BCRT), Charité – Universitätsmedizin Berlin, Berlin, Germany; [4]Department of Experimental Immunology, Amsterdam University Medical Centers, University of Amsterdam, Amsterdam, The Netherlands; [5]Netherlands Brain Bank, Netherlands Institute for Neuroscience, Amsterdam, The Netherlands; [6]Berlin Institute of Health, Berlin, Germany; [7]German Center for Neurodegenerative Diseases (DZNE), Berlin, Germany.*

Multiple sclerosis (MS) is a chronic inflammatory, demyelinating and neurodegenerative disease of the CNS. The pathologic hallmark of MS is the presence of demyelinated lesions throughout the CNS. Active lesions are characterized by myelin loss and the presence of foamy microglia/macrophages containing myelin lipids. Microglia, the resident innate immune cells of the brain, take part in CNS surveillance during homeostasis but are also key players in neurodegeneration and neuroinflammation. In the context of MS, microglia are involved in both demyelination and remyelination, key mechanisms in the development of the disease. Furthermore, recent studies using single cell RNA-seq on mouse models of demyelination have shown distinct transcriptional microglial phenotypes associated to lesions pointing to an important role of microglia in the disease pathogenesis. However, further characterization of microglial phenotypes and their possible contribution to human MS pathogenesis remains to be studied. Here, we applied multiplexed single-cell mass cytometry (CyTOF) to elucidate the different phenotypes of microglia in active lesions and normal appearing white matter (NAWM) of MS patients as compared to control donors. We performed three independent CyTOF measurements with a total of 75 antibodies to analyze myeloid cells isolated from post-mortem human brain. In our study we could confirm consistent changes in the myeloid phenotypes in active lesions as compared to NAWM and control, mainly decreased abundance of homeostatic microglial clusters, with decreased expression of GPR56, P2Y12 and TMEM119 in some lesion associated myeloid cells. We detected an increased abundance in lesion of clusters expressing phagocytic-related and antigen presenting-related markers such as HLADR, CD11c, CD64, CD68, CD47, CD91 and CD172a (SIRPα), among others. In whole, by using multi-dimensional single-cell technology we could unravel previously unidentified myeloid phenotypes associated to MS active lesions, thus emphasizing the complexity of microglia biology in MS.

Chairs: Bertram Bengsch & Henrik Mei

# Dissecting immune cell modulation during therapeutic targeting of CD38 in Systemic Lupus Erythematosus using mass cytometry

**Marie Urbicht[1,3], Lennard Ostendorf[1,2,3], Andreas Grützkau[1], Falk Hiepe[1,2], Tobias Alexander[1,2,3], Henrik Mei[1,3]**

*[1]Deutsches Rheuma-Forschungszentrum Berlin, Germany; [2]Charité Universitätsmedizin Berlin, Department of Rheumatology and Clinical Immunology, Berlin, Germany; [3]equal contribution of first and senior authors*

Systemic Lupus Erythematosus (SLE) is an autoimmune disease characterized by antibodies directed against double-stranded DNA which are secreted by autoreactive plasma cells (PC). Daratumumab (Dara) is a monoclonal anti-CD38 antibody that diminishes neoplastic plasma cells in vivo and is coherently licensed for the treatment of multiple myeloma. Thus, Dara also represents an interesting treatment option in antibody-mediated diseases such as SLE. However, the expression of CD38 in immune cells other than PC has not been systemically studied, especially under conditions of SLE. In this study, we used mass cytometry to characterize the expression of CD38 across innate and adaptive immune cells in peripheral blood in great detail in SLE and monitored immune cell composition during treatment with Dara in two therapy-refractory SLE patients to gain insight in the mechanism(s) of action and secondary targets cells of Dara beyond PC.

SLE and control blood samples were preserved in proteomic stabilizer at -80°C. Samples were Palladium-barcoded, and a 41-parameter mass cytometry panel comprising immune cell-surface markers and phosphoepitope-specific antibodies was established for simultaneous in depth-phenotyping of leukocyte subsets and interrogation of cell type-specific intracellular signaling. Data were analyzed using an R pipeline based on FlowSOM clustering and statistical analysis by SAM. Validation was carried out using manual gating analyses.

Besides peripheral PC, CD38 was expressed in NK cells, plasmacytoid dendritic cells, monocytes, as well as several T and B lymphocyte subsets at varying levels. Consistent with broad immune activation, SLE patients showed increased CD38 expression across many leukocyte subsets. Strikingly, several immune cell subsets expressing CD38 at baseline and in control donors persisted in the treatment with Dara. On levels of cell signalling, we observed that anti-CD38 treatment induced changes in phosphorylation patterns of several key signal transducers, such as STAT proteins, p38 or NFkB. This effect was most prominent in, but not limited to the T cell compartments and paralleled the clinical improvement of the patients.

In summary, CD38 targeting is a promising treatment concept in antibody-mediated diseases. Besides targeting plasma cells, Dara may impact a variety of other innate and adaptive immune cells expressing CD38, including T, B and NK cells subsets, as well as monocytes and plasmacytoid dendritic cells as important sources of inflammatory cytokines. Further analyses are currently being conducted to address whether changes in phosphorylation of intracellular signalling mediators are directly related to CD38 ligation or secondary to decreased disease activity. This study underscores the utility of combined phenotypical and ex vivo signalling analyses by mass cytometry to characterize target expression pattern of therapeutic antibodies as well as to elucidate the immune system's response to treatment.

## Multidimensional analyses of proinsulin peptide-specific regulatory T cells induced by tolerogenic dendritic cells

**Jessica Suwandi[1], Sandra Laban[1], Kincsõ Vass[1], Antoinette Joosten[1], Vincent van Unen[1,3], Boudewijn Lelieveldt[1], Thomas Höllt[1,4], Jaap Jan Zwaginga[1], Tatjana Nikolic[1], Bart Roep[1,2]**

*[1]LUMC, The Netherlands; [2]City of Hope, USA; [3]Stanford, USA; [4]TU, Delft, The Netherlands*

Induction of antigen-specific regulatory T cells (Tregs) in vivo is the holy grail of current immune-regulating therapies in autoimmune diseases, such as type 1 diabetes. Tolerogenic dendritic cells (tolDCs) generated from monocytes by a combined treatment with vitamin D and dexamethasone (marked by CD52hi and CD86lo expression) induce antigen-specific Tregs. We evaluated the phenotypes of these Tregs using high-dimensional mass cytometry to identify a surface-based T cell signature of tolerogenic modulation. Naïve CD4+ T cells were stimulated with tolDCs or mature inflammatory DCs pulsed with proinsulin peptide, after which the suppressive capacity, cytokine production and phenotype of stimulated T cells were analysed. TolDCs induced suppressive T cell lines that were dominated by a naïve phenotype (CD45RA+CCR7+). These naïve T cells, however, did not show suppressive capacity, but were arrested in their naïve status. T cell cultures stimulated by tolDC further contained memory-like (CD45RA-CCR7-) T cells expressing regulatory markers Lag-3, CD161 and ICOS. T cells expressing CD25lo or CD25hi were most prominent and suppressed CD4+ proliferation, while CD25hi Tregs also effectively supressed effector CD8+ T cells.

We conclude that tolDCs induce antigen-specific Tregs with various phenotypes. This extends our earlier findings pointing to a functionally diverse pool of antigen-induced and specific Tregs and provides the basis for immune-monitoring in clinical trials with tolDC.

## Mass cytometry reveals effects of Store-operated Calcium Entry pathway in human intestinal inflammation

**Marilena Letizia[1], Cansu Yerinde[1], Annegret Sand[1], Stephan Schlickeiser[2], Ulrike Kaufmann[3], IBDome-DE Investigators Study group[4], Britta Siegmund[1], Stefan Feske[3], Carl Weidinger[1]**

*[1]Charité Universitätsmedizin Berlin, Germany; [2]Berlin-Brandenburg Center for Regenerative Therapies, Berlin, Germany; [3]Department of Pathology, New York University School of Medicine, New York, NY 10016, USA.; [4]iPATH.Berlin, Charité Universitätsmedizin Berlin, Germany*

Store-operated Calcium Entry (SOCE) represents the major calcium influx pathway in T cells which not only controls the activation and function of lymphocytes, but which also has been implicated in the metabolic homeostasis and survival of murine CD4+ and CD8+ T cells. Conditional knockout mice, in which SOCE signaling components are deleted in T cells, revealed that SOCE is required for the induction of intestinal inflammation in mouse models of colitis. However, the effects of SOCE inhibition have not been studied in the human context of inflammatory bowel disease (IBD) and it remains elusive, which immune cell subset is affected by the pharmaceutical blockade of SOCE. We therefore aim to investigate the effects of SOCE inhibitor BTP-2 on functions and metabolic homeostasis of human lymphocytes isolated from IBD patients.

PBMC and/or lamina propria lymphocytes (LPMC) were isolated from colitis patients undergoing colon resection. Cells were ex -vivo stimulated with Ionomycin/PMA in the presence or absence of BTP-2 and subsequentialy fixed and stored at -80°C until acquisition by mass cytometry. LPMCs were stained using 37 lineage and functional markers targeting B, T, NK or myeloid cells and the resulting flow cytometry standard (FCS) files were analyzed by using cytobank and R/Bioconductor 9 packages. Additionally, Ca2+ influx

measurement and Seahorse analyses were performed in order to assess the metabolic status of immune cell subsets after SOCE inhibition.

Data on B, T, NK, myeloid cells and neutrophils isolated from peripheral blood or colon lamina propria revealed that each immune cell subset harbors a distinctive SOCE-dependent Ca2+ influx rate, suggesting that SOCE might differentially regulate the activation and function of each cell subtype. In particular, CD4+ and CD8+ T cells, B and NK cells as well as monocytes were highly susceptible to extracellular Ca2+ influx, followed by granulocytes. Furthermore, inhibition of SOCE in lymphocytes resulted in impaired metabolic fitness, reduced glycolytic capacity and impaired fatty acid oxidation. Finally, BTP-2 was able to decrease the production of key pro-inflammatory cytokines involved in IBD, including TNFα and IL-17 in lamina propria resident T cells.

Our data revealed for the first time that the cytokine production and the activation of several immune cell subtypes can be modulated by SOCE blockade in human intestinal inflammation, identifying SOCE as a novel therapeutic target in colitis. Moreover, we hope that a wide phenotypical characterization of immune cells via mass cytometry will provide a better insight into positive as well as negative effects of SOCE inhibitors that might interfere with the clinical applicability of SOCE inhibitors for treating IBD.

## Session 4: Workshop: Mass Cytometry Basics and Reagents

Chairs: Henrik Mei & Axel Schulz

## ISAC-Lecture: CyTOF in large studies - Challenges and lessons learned

**Michael Leipold**

*Stanford University School of Medicine, United States of America*

Mass cytometry (CyTOF) has been available for almost 10 years. However, most studies thus far have been relatively small and of short duration. As the technology has matured, there has been increased interest in utilizing it in larger studies, both in size and in longer-duration. Large studies run in a short period of time have different challenges than large studies run over longer periods of time. I will speak about my experiences, challenges, and lessons learned in two studies: first, a large cohort of samples run in a short period of time, and compare that with a cohort of similar size that was acquired and run over the course of several years.

## Single cell gating by cell cycle analysis in mass cytometry

**Idun Dale Rein[1], Heidi Ødegaard Notø[1], Monica Bostad[1], Kanutte Huse[1,2,3], Trond Stokke[1,4]**

*[1]Department of Core Facilities, Institute for Cancer Research, Oslo University Hospital, Oslo, Norway; [2]Department of Cancer Immunology, Institute for Cancer Research, Oslo University Hospital, Oslo, Norway; [3]K.G. Jebsen Centre for B cell malignancies, University of Oslo, Oslo, Norway; [4]Department of Radiation Biology, Institute for Cancer Research, Oslo University Hospital, Oslo, Norway*

A disadvantage of mass cytometry is that aggregates of cells cannot easily be removed while retaining all single cells. Common gating strategies for excluding non-single cell events are based on event length to exclude ion cloud fusions, in addition to DNA content (Ir-intercalator),

and/or a parameter that is related to cell size (e.g. Cisplatin) to exclude aggregates. If cells are barcoded, non-single cell events with different barcodes can be removed. However, cell aggregates, mostly formed during fixation and therefore before the barcoding, as well as ion cloud fusions of cells with the same barcode will not be removed. The use of Ir-intercalator and Cisplatin for removing the remaining non-single cell events is problematic for actively proliferating cells, as two G1 cells has the same DNA content and about the same size as a G2 or mitotic cell. Gating based on a narrow region in any marker that is related to DNA content or size around the diploid (G1) cells will exclude cells late in the cycle. A too wide region for gating will result in inclusion of aggregates. These questions were addressed by us in a manuscript in revision in Cytometry A.

In the submitted work we developed an analysis pipeline where unsupervised clustering by FlowSOM was performed as initial clean-up and gating of the data, resulting in separate meta-clusters identified as the major cell cycle phases in addition to dead and apoptotic cells, debris and some non-single cell events. Each cell cycle phase was then individually gated to exclude ion cloud fusions and aggregates within cell size-homogeneous groups, thereby avoiding inclusion of small aggregates or exclusion of cells late in the cycle. The minimum panel to distinguish the cell cycle phases consisted of Ir-intercalator (DNA content), IdU (S phase), anti-pS28HistoneH3 (mitosis), anti-CDT1 (G1 phase) and anti-Geminin (non-G1 phases).

The new Gaussian parameters that are acquired in the Helios simplifies the removal of ion cloud fusions, and an alternative pipeline is presented here that includes using these parameters. As the Gaussian parameters width, residual, offset and center describe the ion cloud-pulse shape, any event with a non-gaussian curve can be excluded, which will be the case for ion cloud fusions. The pipeline suggested here includes the following steps:

1. Normalization and debarcoding: Will remove hetero-barcoded events (mostly ion cloud fusions)
2. Use of the Gaussian parameters to remove remaining ion cloud fusions.
3. FlowSOM clustering and annotation of cell cycle phases. This step will also remove some aggregates.
4. Cell cycle phase-specific single cell gating (based on cell size). This step will remove the rest of the aggregates.
5. Visualization of the cell cycle phases in e.g. viSNE plots.

When performed on cells stained with the cell cycle panel, this analysis pipeline successfully identified ion cloud fusion events, debris, dead/apoptotic cells, aggregates and the major cell cycle phases. The presented cell cycle panel and analysis pipeline thus enables single-cell analysis also in samples with cells in all cell cycle phases at the same time as any additional channels in the panel are open for phenotyping and/or cell cycle-resolved expression or modification analysis.

## High-Dimensional Single-Cell Mass Cytometry Analysis in Murine Models of Alzheimer´s Disease & Tauopathy upon PD-L1 immune checkpoint blockade

**Tomer Meir Salame[1], Javier Maria Peralta Ramos[2], Giulia Castellani[2], Hila Ben Yehuda[2], Michal Arad[2], Tommaso Croese[3], Michal Schwartz[2]**

*[1]Flow Cytometry Unit, Life Sciences Core Facilities, Weizmann Institute of Science, Rehovot, Israel; [2]Department of Neurobiology, Weizmann Institute of Science, Rehovot, Israel; [3]Clinical Neuroimmunology Unit, Institute of Experimental Neurology, Division of Neuroscience, San Raffaele Scientific Institute, Milan, Italy*

Alzheimer's disease (AD) is associated with accumulation of amyloid-ß plaques and aggregation of tau tangles. In previous reports we have shown that, in both murine models of amyloidosis, AD (5XFAD) and of tauopathy (DM-hTAU), that targeting of the Programmed Death (PD)-1/PD-L1 axis improves the cognitive performance during assessment of short-term spatial and working memory by harnessing the immune system through blocking of the inhibitory immune checkpoint-mediated. Here we performed a full kinetic and dynamic characterisation of resident versus systemic cells recruited into the CNS, which could potentially mediate an important function in disease modification, unravelling their molecular signature by single-cell mass cytometry (CyTOF). CyTOF has enabled us unbiased simultaneous analysis, providing high-resolution proteomic profiles of states and dynamics of microglia and peripheral recruited leukocytes, revealing a remodeling of blood and brain immune compartments in a time-dependent manner upon immunotherapy in AD and dementia. This study provides an approach for deep immune profiling across disease progression after immunotherapy, covering identity, function and immune regulation, while delving deeper in our overall comprehension of the immune landscape in neurodegenerative diseases.

## A robust human immunophenotyping workflow using CyTOF technology coupled with Maxpar Pathsetter, an automated data analysis software: The Maxpar Direct Immune Profiling Assay

**Stephen K.H. Li[1], Daniel Majonis[1], C. Bruce Bagwell[2], Benjamin C. Hunsberger[2], Thirumahal Selvanantham[1], Greg Stelzer[3], Vladimir Baranov[1], Mayur V. Bakshi[4], Olga Ornatsky[1]**

*[1]Fluidigm Canada Inc., Markham, Ontario, Canada; [2]Verity Software House, Topsham, Maine, United States; [3]Fluidigm Corporation, South San Francisco, California, United States; [4]Fluidigm GmbH, Munich, Germany.*

Immune profiling is the practice of identifying and quantifying immune populations and their features. Performing immune profiling over time is referred to as immune monitoring. Techniques for immune monitoring in blood are commonly applied in translational and clinical research settings to provide phenotypic understanding of immune states prior to and following treatment. The diversity of immune cell populations demands a high-parameter technique to more fully and efficiently quantify these changes. Mass cytometry, which utilizes CyTOF® technology, is a single-cell analysis platform that uses metal-tagged antibodies and can resolve over 50 markers in a single sample tube without the need for compensation. It is an ideal solution for routine enumeration of immune cell populations. However, development of a robust, highly

multiplexed assay requires panel optimization as well as standardization of instrument setup and an easy-to-use yet reliable data analysis solution.

We have developed a sample-to-report solution for human immune profiling using mass cytometry, the Maxpar® Direct™ Immune Profiling System. It includes an optimized 30-marker immune profiling panel provided in a dry, single-tube format, protocols for human whole blood and PBMC staining, a Helios™ data acquisition template, and Maxpar Pathsetter™ software for automated data analysis. The Pathsetter software analyzes FCS 3.0 files generated with the kit and automatically reports cell counts and percentages for 37 immune cell types. It also reports quality metrics such as staining assessment. Pathsetter produces graphical elements such as dot plots and a Cen-se'™ map (t-SNE variant).

Here we present assay analytical validation data on repeatability, reproducibility, software precision, and software accuracy*. We also present a performance comparison between dry and liquid formulations of the same antibodies and clones*. This assay provides a robust, complete solution for broad immune profiling using mass cytometry that reduces sources of variability and subjectivity in sample preparation and data analysis.

## Characterization of cell composition from peripheral blood from degenerative and chronic inflammatory arthritis patients

**Daniela Paclik[1], Nadine Biesemann[2], Matthias Pumberger[3], Henri Mei[4], Stephan Schlickeiser[1,5], Axel Ronald Schulz[4], Birgit Sawitzki[1]**

*[1]Charité - Universitätsmedizin Berlin, Institut für Medizinische Immunologie; [2]Sanofi R&D Immunology and Inflammation Therapeutic Area, Type 1/17 Inflammation and Arthritis Cluster; [3]Charité - Universitätsmedizin Berlin, Centrum für Muskuloskeletale Chirurgie; [4]German Rheumatism Research Centre Berlin (DRFZ), Mass Cytometry and Immune Monitoring; [5]Berlin-Brandenburg Centrum für Regenerative Therapien (BCRT)*

Introduction: Rheumatoid arthritis (RA) and spondyloarthritis (SpA) are autoimmune diseases leading to joint and bone destruction due to uncontrolled inflammatory processes. Although, both diseases share target organs, they differ in their responses to cytokine targeting therapy such as IL-17A inhibition. Thus, it remains unclear whether RA and SpA pathology are driven by distinct immune and non-immune cell subsets or whether overlapping profiles can be observed.

Aim: We compare alterations in cellular composition of local tissue and peripheral blood between RA and SpA patients. Using mass cytometry we assess the cellular composition in PBMCs, leukocytes and bone tissue (knee and spine) of patients with RA and SpA undergoing surgery for either total knee replacement or spinal fusion, respectively.

Patients suffering from degenerative diseases and undergoing identical surgical procedure serve as controls as bone tissue from healthy non-diseased controls is not available. In a first step, we thus analyzed whether PBMC samples of patients suffering from degenerative diseases have a different cellular composition to those of healthy controls.

Methods: PBMCs from healthy volunteers (HC; n=11; median age 47), degenerative patients undergoing surgery for total knee replacement (DegK; n=9; median age 75) or undergoing spine surgery (DegS; n=9; median age 76) were analyzed by mass cytometry. The staining panel comprised 44 markers allowing identification and characterization of different T cell, B cell, NK cell, ILC and dendritic cell subsets. The acquired data sets were analyzed in Cytobank using FlowSOM and citrus algorithm. Statistical analyses were performed by Wilcoxon-Mann-Whitney test and Dunn´s comparison for multiple comparisons.

Results: Statistical evaluation of 100 PBMC FlowSOM clusters determined 4 significantly different T cell clusters between HC and DegK and 6 between HC and DegS. In the monocyte compartment 4 clusters were significantly different between HC and DegK and 5 clusters between HC and DegS. Most of the T cell subsets that were more abundant in DegS and DegK patients showed a marker profile of differentiated cells. Since DegK and DegS patients were significantly older than healthy controls we repeated the analysis with age-matched samples. We ended up with just 6 samples per group and a median age of 55 (HC) and 66 (DegK and DegS). Using the above approach, we could not detect any significantly different cell clusters, neither in the T cell nor in the monocyte compartment, between the groups using FlowSOM (100 clusters) and citrus clustering (minimum cluster-size threshold of 5%).

Conclusions and limitations: The cellular composition within PBMCs of patients with degenerative diseases and healthy controls is similar making degenerative patients valuable controls for future investigations. However, due to the small cohort size we cannot completely rule out existing differences. Our results stress the relevance of age-matching of control samples in deep immune profiling studies.

## Exploring Healthy and Tumor Tissue Microenvironment with Immuno-Oncology Markers Using Multiplexed HyperionTM Imaging System

**Dongxia Lin[1], Jeremy Sarnecky[1], Eric Swanson[2], Christina Loh[3], Mary-Kay Lippert[4], Marc Reudelsterz[5]**

*[1]Reagents Operation, Fluidigm Corporation, South San Francisco, CA, USA; [2]Field Application Support, Fluidigm Canada Inc., Markham, ON, Canada; [3]Reagents Development, Fluidigm Canada Inc., Markham, ON, Canada; [4]Reagents Operation, Fluidigm Canada Inc., Markham, ON, Canada; [5]Field Support, Fluidigm Germany GmbH*

To power immuno-oncology discovery, it is highly beneficial to explore healthy and tumor tissues with immuno-oncology markers using multiplexed analysis. The Hyperion™ Imaging System uses novel technology for tissue imaging that enables multiplexed analysis of protein expression in a single tissue sample. This methodology uses tissue sections stained with a cocktail of antigen-specific antibodies conjugated to different metal isotopes. In this study, we demonstrate how to generate high-parameter images with highly relevant immuno-oncology markers on the Hyperion Imaging System.

To detect multiple markers in one panel, we optimized the tissue staining protocol for signal detection and tissue preservation. For formalin-fixed, paraffin-embedded (FFPE) tissue staining, antigen retrieval conditions (temperature and incubation time) were optimized. We determined that antigen retrieval conditions of 96 °C for 30 minutes in basic retrieval solution enabled detection of nuclear markers such as FoxP3, along with other surface and cytoplasmic markers. To verify these methods, we also generated equivalent data to compare these results with immunofluorescence on FFPE tissue sections and examined co-localization and anti-localization of the antibodies with previously verified counter stains.

Using these optimized staining protocols, we generated images from various normal and tumor tissues (including diffuse large B cell lymphoma, colon adenocarcinoma, and bladder urothelial carcinoma) to show a combination of 5 structural, 1 cancer, 3 nuclear, and 18 immuno-oncology markers simultaneously. Together with other tissue architectural details, different immune cell types were identified in both normal and tumor tissues. This image resolution allowed for the visualization of proteins in the membranous, cytoplasmic, and nuclear cell compartments. Therefore, our data demonstrate that the Hyperion Imaging System provides a high-parameter imaging solution at subcellular resolution to charac-

terize the immune repertoire in the tumor microenvironment.

## Immune profiling of human Peyer´s Patches in patients of inflammatory bowel disease

**Yasmina Rodríguez Sillke[1,2], Ulrich Steinhoff[3], Christian Bojarski[1], Michael Schumann[1], Donata Lissner[1], Désirée Kunkel[4], Federica Branchi[1], Rainer Glauben[1], Britta Siegmund[1]**

*[1]Medical Department (Gastroenterology, Infectious Diseases, Rheumatology) Campus Benjamin Franklin, Charité - Universitätsmedizin Berlin, Berlin, Germany; [2]Institute of Nutrition, University of Potsdam, Nuthetal, Germany; [3]Institute for Medical Microbiology and Hygiene, University of Marburg, Marburg, Germany; [4]Flow & Mass Cytometry Core, Campus Virchow-Klinikum, Charité - Universitätsmedizin Berlin, Berlin, Germany*

1. Introduction

One of the hallmarks of inflammatory bowel disease (IBD) is a dysregulation of the intestinal immune system. Although nutritional therapy is effective, little is known about its mechanism. The immune system is linked to food antigens through the Peyer´s patches (PP) in the ileum, which are essential for oral tolerance induction. Murine data indicate that food antigens induce an activation and subsequent apoptosis of the CD4+ T-cells in the PP thus maintaining the healthy balance of the mucosal immune system.

2. Objectives

Human PP cells from the terminal ileum of IBD patients and healthy controls were analysed for their phenotype and fate.

3. Materials & methods

Human fresh PP cells were characterized between Crohn´s disease (CD), Ulcerative colitis (UC) patients and healthy controls. Apoptosis measurements and basic phenotyping were performed via flow cytometry. For a deeper analysis, PP cells were analysed via mass cytometry (CyTOF2 – Helios).

4. Results

PP activated B-cells revealed a reduction in active CD and UC. Moreover, PP CD8+ T-cells were increased in CD and UC compared to healthy controls, especially effector memory T-cells expressing T-bet and Bcl2. In contrast, PP CD4+ T-cells were similar in all patients groups. However, within the CD4+ T-cells, CD showed an increase of central memory T-cells and a reduction of effector memory T-cells compared to healthy controls. Furthermore, PP CD4+ T-cells of CD patients revealed a significantly reduced apoptotic rate compared to UC patients and healthy controls. Further characterization identified a decrease of Helios+FoxP3- in CD patients, described as diet-specific T-cells undergoing apoptosis.

5. Conclusion

In the healthy human gut, food-activated CD4+ T-cells in PPs exhibited a pro-apoptotic phenotype characterized by the transcription factor Helios. In contrast, low Helios+ FoxP3- expression and reduced apoptosis were observed in PP CD4+ T-cells of patients with IBD, suggesting that activation and subsequent death of food-reactive T-cells is a hallmark of intestinal homeostasis.

## Characterization of the liver and lung stromal cells populations by mass cytometry

**Aleix Rius Rigau[1], Simon Rauber[1], Oisin Lancaster[2], Meik Kunz[2], Andreas Ramming[1], Jörg Distler[1]**

*[1]Department of Internal Medicine 3 - Rheumatology and Immunology, Friedrich-Alexander-University Erlangen-Nürnberg (FAU) and University Hospital Erlangen, Erlangen 91054, Germany; [2]Lehrstuhl für Medizinische Informatik, Universität Erlangen-Nürnberg, D-91058 Erlangen-Tennenlohe*

An organ or tissue can be divided in three compartments: immune cells, parenchyma and stroma. The parenchyma is the tissue responsible for the organ function (the hepatocytes in the liver or lung epithelial cells in the lung). On the other hand, the term "stroma" is used to describe the supporting substance of tissues and its principle role is to maintain the microenvironment required by the parenchyma.

Stroma cells comprise different cell populations and subpopulations, each of them with different functions, which have not been fully elucidated so far. Nevertheless, a crosstalk between stromal and immune cells has been described and the impairment of this communication can lead to pathogenic conditions. For example: fibroblast can act as immune sentinels (with some capacity for phagocytosis and antigen presentation) and also releasing chemokines and cytokines; the endothelial cells can change their phenotype to increase the immune cell transmigration through homing molecules and chemokines receptors expression.

The aim of this project is to phenotypically characterize stromal cells in liver and lung tissues from mice under physiological condition. For this reason we design a 37 metals CyTOF panel where we can study all the stromal cells populations and the pathways implicated in different diseases.

We first adopted negative markers for immune cells and macrophages, epithelial cells, lung epithelial cells and hepatocytes: CD45 and CD68, E-cadherin, Claudin-18 and ASGPR, respectively. Then we defined as "Stromal cells" the remaining population after the negative selection.

Applying the FlowSOM algorithm to the stromal cells, we can define the Lymphatic endothelial cells (CD31+ Podoplanin+), hepatic sinusoidal endothelial cells / lung arterial endothelial cells (CD31+ sca-1+), mesenchymal stem cells (sca-1+ CD31-), fibroblast (Podoplanin+ CD31-), pericytes (α-sma+, PDGFRβ+) and smooth muscle cells (α-sma+ PDGFRβ-) as well as their subpopulations.

This panel and the FlowSOM high-dimensionality analysis allowed us to study deeply the importance of the stromal cells in different diseases like cancer, rheumatoid arthritis and another autoimmune disorders, fibrotic conditions and wound healing. The improvement of our knowledge in this field can emerge new treatments or strategies to improve the current ones.

## Single cell mass cytometry of A549 non-small cell lung cancer cells reveals complexity of the in vivo model and three-dimensional cell cultures over the Petri-dish

**Robert Alfoldi[1,2], Jozsef A. Balog[3], Nora Farago[4,5,6], Miklos Halmai[3], Edit Kotogany[3], Lajos I. Nagy[4], Lilana Z. Feher[4], Laszlo G Puskas1[,3,4], Gabor J. Szebeni[3,6]**

*[1]Avicor Ltd., Also kikoto sor 11/D, Szeged, Hungary; [2]Astridbio Technologies Ltd., Szeged, Hungary; [3]Laboratory of Functional Genomics, Biological Research Centre, Temesvari krt. 62. Szeged, Hungary; [4]Avidin Ltd., Also kikoto sor 11/D, Szeged, Hungary; [5]Research Group for Cortical Microcircuits, Department of Physiology, Anatomy and Neuroscience, University of Szeged, Kozep fasor 52, Szeged, Hungary; [6]Department of Physiology, Anatomy and Neuroscience, Faculty of Science and Informatics, University of Szeged,*

*Kozep fasor 52, Szeged, Hungary*

Single cell genomics and proteomics revolutionized cancer discovery. These advancements with the combination of innovative three-dimensional (3D) cell culture techniques can open new avenues toward the understanding of intra-tumor heterogeneity. To achieve high resolution measurement of cellular features, single cell mass cytometry was used in our laboratory combining advantages of the single cell resolution of traditional fluorescence-based flow cytometry with the multiplexicity of inductively coupled plasma-mass cytometry. Here, we addressed the single cell mass cytometric characterization of lung cancer markers under different conditions: two-dimensional (2D), carrier-free or bead-based 3D culturing and in vivo.

Investigation of the proliferation, viability and cell cycle phase distribution were performed. Gene expression analysis resulted the selection of markers which were overexpressed either in vivo or in long-term 3D cultures: TMEM45A, SLC16A3, CD66, SLC2A1, CA9, CD24 or repressed: EGFR. Additionally, TRA-1-60, pan-keratins, CD326, Galectin-3 and CD274 with known clinical significance were investigated at single cell resolution.

Multidimensional single cell proteome profile revealed that 3D (Cytodex3 and Nutrisphere) cultures represent a transition from 2D to in vivo situation by intermediate marker expression of TRA-1-60, TMEM45A, pan-keratin, CD326, MCT4, Gal-3, CD66, GLUT1, CD274. In 3D systems CA9, CD24, EGFR were exposed to the cell surface superior to in vivo. However, all twelve markers drew the map of in vivo A549 lung cancer cells as a different islet from the population of cells of 2D and 3D samples with a characteristic protein pattern reflecting intra-tumor heterogeneity. Therefore, 3D cultures of NSCLC cells bearing more putative cancer targets should be used in drug screening as the preferred in vitro technique rather than the Petri-dish.

Funding: 2017-1.3.1-VKE-2017-00028 (Avicor Ltd.) and GINOP-2.3.2-15-2016-00001 (BRC). Gábor J. Szebeni was supported by János Bolyai Research Scholarship of the Hungarian Academy of Sciences (BO/00139/17/8) and by the UNKP-19-4-SZTE-36 New National Excellence Program of the Ministry for Innovation and Technology.

## Identifying immune subsets reflecting response to mesenchymal stromal cell therapy in steroid-refractory acute graft-versus-host disease

**Sandra Laban, Jessica S. Suwandi, Jelske Borst, Anna-Sophia Wiekmeijer, Sanne Hendriks, Bart O. Roep, Jaap Jan Zwaginga, Willem E. Fibbe, Koen Schepers, Astrid G.S. van Halteren**

*Leiden University Medical Center, The Netherlands*

Graft-versus-host disease (GvHD) is a life-threatening complication following transplantation of allogeneic hematopoietic stem cells. Treatment with mesenchymal stromal cells (MSCs) is a candidate therapy for second line treatment of patients with steroid-refractory GvHD. In a prospective cohort, pediatric patients with GvHD were treated with MSC infusion and peripheral blood samples were collected and cryopreserved at different time points: 1 week before, 1 week after and 4 weeks after treatment. In this study, we will determine the effect of MSC therapy on subsets of cells of the innate and adaptive immune system in peripheral blood. For this purpose, we will include patients that completely resolved clinical symptoms of GvHD after MSC infusion (n=11), and patients that did not respond to therapy (n=5). As a control, we will include blood samples from healthy pediatric bone marrow donors and children that received allogeneic hematopoietic stem cell and did not develop GvHD. To analyze these blood samples, we developed and validated two mass cytometry panels with a total of 78 metal-tagged antibo-

dies, each detecting 14 overlapping and 25 unique markers. The first panel focuses on T cells and includes T cell markers as well antibodies targeting homing molecules, while the second panel includes markers to distinguish subsets of B cells, NK cells, myeloid cells and innate lymphoid cells. To reduce batch effects, we implemented live cell barcoding with anti-ß2M antibodies conjugated with palladium metals enabling multiplexing of six samples. By comparing GvHD patients responding to MSC therapy with non-responding patients before and after MSC infusion, we expect to gain novel insights in the working mechanism of MSCs in alloimmunity and find immune signatures predictive of response to MSC therapy.

## High dimensional mass cytometry analysis reveals a novel signature of IL-17 producing CD8 T cells that accumulate in active inflammatory bowel disease

**Anna-Maria Globig[1,2], Anna Hipp[1], Patricia Otto-Mora[1], Maximilian Heeg[2,3], Henning Schwacha[1], Vesselin Tomov[4], Robert Thimme[1], Peter Hasselblatt[1], Bertram Bengsch[1,5]**

*[1]Department of Medicine II, Gastroenterology, Hepatology, Endocrinology, and Infectious Diseases, University Medical Center Freiburg, Faculty of Medicine, Freiburg, Germany; [2]Faculty of Medicine, University of Freiburg, Berta-Ottenstein-Programme, Freiburg, Germany; [3]Institute for Immunodeficiency, Center for Chronic Immunodeficiency, University Medical Center Freiburg, Faculty of Medicine, Freiburg, Germany; [4]Department of Medicine, Division of Gastroenterology, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA; [5]Signalling Research Centres BIOSS and CIBSS, University of Freiburg, Freiburg, Germany*

Background
The pathogenesis of IBD has previously mainly been associated with a dysregulation of CD4 T cell responses, and active disease is linked to an induction of pathogenic IL-17 producing T helper cells. Importantly, numerous subpopulations of CD8 T cells can also produce IL-17 (Tc17 cells). However, it remains unclear whether these populations are linked to disease activity, and whether Tc17-derived IL-17 is produced by conventional αß T cells or unconventional T cells. IBD-specific Tc17 dynamics could play a role as novel biomarkers or therapeutic targets of active IBD.

Methods
We isolated lymphocytes from the peripheral blood and intestinal tissue of IBD patients (n=56) and performed a detailed analysis of CD8 T cell phenotype and function using high-parametric flow cytometry and mass cytometry in combination with algorithm-aided bioinformatic analysis.

Results
We observe a significant increase in IL-17 production by CD8 T cells in active IBD, primarily produced by conventional CD8 T cells. Unconventional T cell subsets (e.g. MAIT cells, gammadelta T cells and NKT cells) represented only ~30% (peripheral blood) or ~25% (intestinal tissue) of IL-17 producing CD8 T cells (Tc17). Mass cytometric uMAP and FlowSOM analysis identified Tc17 cells as a distinct cell population within the intestinal CD8 T cell compartment that could be further subdivided into 3 subsets which share expression of phenotypic markers such as CD6, CD39, CD69 and PD1 and a low expression of CD27. This novel signature was validated in a separate cohort of IBD patients. Moreover, at initial IBD diagnosis, the IL-17 signature is associated with flare free survival in a retrospective cohort analysis based on published transcriptome data.

Conclusion
Our data indicate that conventional IL-17 producing CD8 T cells are a distinct T cell population is linked to IBD activity. The identification of a novel IL-17 CD8 signature may help guide treatment decisions as a biomarker and for immunotherapeutic approaches.

# Tight control Spondylarthritis and Mass Cytometry: Monitoring immune profiles during treatment compared to HLA-B27 controls reveals global changes and normalisation over time

**Hester Koppejan[1], Marjolijn Hameetman[1], Vincent van Unen[1,2], Guillaume Beyrend[1], Tamim Abdelaal[1,3], Rene Toes[1], Floris van Gaalen[1]**

*[1]Leiden University Medical Center, The Netherlands; [2]Stanford University School of Medicine, USA; [3]Delft University of Technology, The Netherlands*

Introduction:
Axial Spondylo Arthritis, also known as spondyloarthritis (SpA), is a rheumatic inflammatory disease. SpA manifests mainly in the axial joints though enthesitis, dactylitis, psoriasis, uveitis and inflammation of peripheral joints are often present as well. Tight control Spondylarthritis (TiCoSpA CT03043846) focusses on reducing disease activity (ASDAS) within a given timeframe according to a strict treatment protocol: if ASDAS exceeds 2.1 at the time of evaluation, treatment is intensified. Each patient has a follow-up up to 1 year and blood is drawn at baseline, 24 weeks and 48 weeks.

SpA research has implicated various immune cell types to be involved in the pathophysiology, such as IL-17 producing cells, TNFa producing cells and even IL-23 producing cells, however a clear broad immune profile is still lacking. To this end we used a 35-marker Mass Cytometry panel to gain more insight into the immune profile of early SpA patients compared to HLA-B27 controls. Additionally, we used both follow-up timepoints to evaluate the changes in the immune profile during treatment.

Design:
Thirty-five cell markers were simultaneously assessed by Mass Cytometry in 9 early, untreated SpA patients at baseline, 24 weeks and 48 weeks. Next to the patient samples, 7 HLA-B27 matched controls were included. All samples were freshly stained and acquired, to prevent any bias of cryopreservation. To monitor technical variation, a separate cryopreserved PBMC reference sample was included. Normalised FCS files were processed to obtain single/live/CD45+ cells for further analysis. CD45+ cells of both baseline and controls were sample-tagged, hyperbolic ArcSinh-5 transformed and simultaneously subjects to dimension reduction (hSNE) and Gaussian Mean shift clustering in Cytosplore (default). Cluster frequency, comparing baseline to control, was analysed using Cytofast in R. Additionally, this initial clustering was used to train a linear discriminant analyser classifier. The classifier was used to cluster newly acquired samples, including both week 24 and week 48.

Results:
Immunoprofiling baseline and control samples revealed significant differences in cluster frequencies related to several immune compartments, including CD4 and CD8 T cells, NK T cells, B cells, CD16+ NK cells and monocytes. PCA indicated a clear separation of both groups based on their immune profile, clustering patients and controls separately. Using the trained classifier, we were able to monitor these clusters overtime during treatment. Interestingly, most of these clusters seemed to normalize during treatment, compared to the frequency within HLA-B27 controls, e.g. Th17 cells (defined as CD4+CD45RO+CCR6+) and CD38+ B cells and monocytes were reduced over time. Interestingly, NK T cell and CD16+ NK cell frequency was not affected by treatment and remained elevated at all three time points compared to controls.

Conclusion:
Using Mass Cytometry, we were able to obtain a broad immune profile of early, untreated SpA patients with active disease. Comparing their profile to HLA-B27 controls revealed differences in several immune compartments. Interestingly, tracking these clusters during treatment indicated these differences normalize over time as disease activity decreases. Our findings of this pilot study suggest that tight control has an effect both on a clinical and an immunological level.

## Novel plasma cells subsets of the human bone marrow

**Antonia Niedobitek, Axel Schulz, René Riedel, Mir-Farzin Mashreghi, Sarah Grässle, Eva Holzhäuser, Sarah Gillert, Henrik Mei**

*Deutsches Rheuma-Forschungszentrum (DRFZ), Berlin, a Leibniz-Institute, Germany*

Long-lived plasma cells (PC) comprise a fundamental part of humoral immunity and immune memory. While persistent protective antibody titers are desired for healthy immune defense, autoreactive plasma cell depletion is pursued in the treatment of antibody-mediated diseases. Despite their relevance in immunity and autoimmunity, the phenotype and regulation of long-lived PC remain obscure. In humans, CD19-negative PC have been shown to contain long-lived PC. Moreover, this PC compartment is enriched in the bone marrow compared to other systemic tissues and display a pro-survival, mature phenotype.

*We have found substantial CD19- and CD19+ bone marrow plasma cell (BMPC) subsets with differential expression of CD45 and CD56, raising the question whether this heterogeneity may reflect different lifespans of PC. CD45 and CD56 are of particular interest in the context of PC longevity due to their potential functions in negative regulation of signaling thresholds, and cell positioning and adhesion, respectively. By Elispot we confirm that CD19+/- CD45+/- CD56+/- BMPC are functionally intact antibody-secreting cells and display different antibody isotype compositions. Differential gene expression analysis of these subsets identifies transcriptional differences which may potentially regulate distinct BMPC lifestyles. To address to localization and cellular environments of CD19- PC, CD19+, and novel PC subsets in human BM, we are establishing multiplexed bone marrow histology via imaging mass cytometry. Importantly, residence in distinct cellular environments has the potential to support different degrees of PC durability.*

The present data provide first indications of a substantial phenotypical heterogeneity of human BMPC. Understanding the interrelation of mature human PC subsets may provide new therapeutic approaches to edit the BMPC repertoire in infection and autoimmunity.

## Immunophenotyping of Drosophila Hemocytes by Single Cell Mass Cytometry

**József Á. Balog[1,2], Viktor Honti[3], Éva Kurucz[3], Beáta Kari[3], László G. Puskás[1], István Andó[3], Gábor J. Szebeni[1,4]**

*[1]Laboratory of Functional Genomics, Biological Research Centre, Szeged, Hungary; [2]University of Szeged, Ph.D. School in Biology, Szeged, Hungary; [3]Immunology Unit, Biological Research Centre, Szeged, Hungary; [4]Department of Physiology, Anatomy and Neuroscience, Faculty of Science and Informatics, University of Szeged, Hungary*

Drosophila melanogaster (Dm) as an arthropod from Protostomia bear only innate immune components with different cellular and humoral effectors. Due to the lack of the adaptive immune system of Dm, it may seem to be difficult to compare with mammals. However, Dm became an important multicellular model organism with highly conserved biochemical pathways at subcellular level and with a wide inventory of genetic mutants. In order to unravel phenotypic heterogeneity of different mutants at protein level, mass cytometry, a multiplex state-of the art technology with single cell resolution has been optimized for Dm in our laboratory.

We have metal-conjugated six antibodies against cell surface antigens (H2, H3, H18, L1, L4, P1), against two intracellular antigens (3A5, L2) and one anti-IgM for the detection of L6 surface antigen, as well as one anti-GFP for the detection of crystal cells in the immune induced samples. A comparative FACS and SCMC analysis with anti-

bodies to discriminative cell surface and intracellular antigens expressed in hemocyte subsets of both IgG and IgM type showed a good accordance of results, in terms of positivity on hemocytes. Further, we investigated the antigen expression profile of single cells and hemocyte populations in naive, in immune induced states, in tumorous mutants (hopTum bearing a driver mutation and l(3)mbn1 carrying deficiency of a tumor suppressor) as well as in stem cell maintenance defective hdcΔ84 mutant larvae. Multidimensional analysis enabled the discrimination of the functionally different major hemocyte subsets, lamellocytes, plasmatocytes, crystal cell, and delineated the unique immunophenotype of the mutants. Our results demonstrated for the first time, that mass cytometry, a recent single cell technology coupled with multidimensional bioinformatic analysis represents a versatile and powerful tool to deeply analyze the regulation of cell mediated immunity of Drosophila at protein level.

Funding: Gábor J. Szebeni was supported by János Bolyai Research Scholarship of the Hungarian Academy of Sciences (BO/00139/17/8) and by the UNKP-19-4-SZTE-36 New National Excellence Program of the Ministry for Innovation and Technology.

## Mass cytometry combined with computational data mining reveals a polymorphous immune cell signature of active rheumatoid arthritis

**Axel Ronald Schulz[1], Tyler Burns[1], Silke Stanislawiak[1], Sabine Baumgart[1], Vera Bockhorn[1], Julia Patermann[2], Sandra Burger[2], Andreas Krause[2], Andreas Grützkau[1], Henrik Mei[1]**

*[1]Deutsches Rheuma-Forschungszentrum (DRFZ), Berlin, a Leibniz-Institute, Germany; [2]Immanuel Krankenhaus Berlin, Klinik für Innere Medizin, Abteilung Rheumatologie und Klinische Immunologie, Berlin, Germany*

Background

Innate and adaptive immune mechanisms drive the pathogenesis of rheumatoid arthritis (RA) and are targets of approved therapies. However, not all patients can be appropriately treated, which defines the need for additional therapeutic concepts combined with personalized treatment. At the same time, a systematic assessment of immune cell dysregulation in the patients` blood that may provide insight into common and individual immune pathology features is lacking.

Methods

We here employed 43-dimensional mass cytometry (CyTOF technology) to deeply profile PBMC in 34 patients with active RA vs. 31 age/gender-matched controls, permitting the automated identification of 60 global PBMC, 80 T cell, and 50 B cell populations by a nested FlowSOM clustering / hierarchical gating approach.

Results

Active RA was characterized by diminished frequencies of MAIT, gd T cell and regulatory T cell, IgA+ and IgM+ memory B cell clusters, while the frequency of CD14highCD16low classical monocytes was significantly increased (SAM analysis, FDR=0.05). While MAIT and gd T cell frequencies were inversely correlated with serum Crp (r=-0.55 and -0.56, p<0.001), IgA+ memory B cells inversely correlated with DAS28 values (r=-0.34, p=0.04), suggesting that some, potentially mucosal-related, components of the RA immune signature are associated with disease activity. Furthermore, computational data mining by FlowSOM, Citrus and CellCNN consistently revealed significantly lower detection of the inflammatory chemokine receptor CXCR3 in RA patients across different T cell, B cell, NK cell, pDC and mDC subsets. We could show that only in RA patients CXCR3 expression levels inversely correlated with peripheral abundance of CXCR3 expressing lymphocyte subsets, which might indicate a continuous migration of CXCR3 expressing to the inflamed tissue.

Conclusion

In this study, we established a multi-component immune cell fingerprint of active RA featuring

aberrations of innate and adaptive immune cells. This immune cell reference map of RA will serve for comparison with data from other autoimmune diseases and longitudinal profiling of patients during therapy.

## Intrinsic heterogeneity of human non-small cell lung cancer cells under the hand-glass of single cell mass cytometry

**Patrícia Neuperger[1,2], József Á. Balog[1,2], József Furák[3], Edit Kotogány[1], Klára Szalontai[4], Imola Mán[5], László G. Puskás[1,5], Gábor J. Szebeni[1,6]**

*[1]Laboratory of Functional Genomics, Biological Research Centre, Szeged, Hungary; [2]Doctoral School of Biology, University of Szeged, Szeged, Hungary; [3]Department of Surgery, University of Szeged, Szeged, Hungary; [4]Csongrád County Hospital of Chest Diseases, Deszk, Hungary; [5]Avidin Ltd, Szeged, Hungary; [6]Department of Physiology, Anatomy and Neuroscience, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary*

Lung cancer is the main cause of cancer related death worldwide, evertheless among others non-small cell lung cancer (NSCLC) is the most common type with 85% of all lung cancers. Since the intratumor heterogeneity has been reported to be responsible for the major difficulty in the treatment of lung cancer patients, the heterogeneity of non-small cell lung cancer cells has been investigated by single cell mass cytometry (SCMC).

Human NSCLC cell lines A549, H1975 and H1650 were synchronized by sublethal concentration of hydroxyurea in order to exclude heterogeneity associated with different cell cycle phases. Hypoxic microenvironment was modeled by deferoxamine (DEF) treatment of A549, H1975 and H1650 NSCLC cells. Additionally, human primary adenocarcinoma and non-involved healthy lung tissue biopsies were homogenized to single cell suspension for mass cytometric analysis. Gene expression changes were measured by quantitative real-time PCR (qRT-PCR) of DEF treated NSCLC cells vs. normoxia and human primary adenocarcinoma cells vs. healthy lung tissue.

Although the synchronization showed the absence of G2/M cell cycle phase, the expression pattern of 14 markers: GLUT1, MCT4, CA9, TMEM45A, CD66, CD274 (PD-L1), CD24, CD326 (EpCAM), Pan-Keratin, TRA-1-60, HLA-A,B,C, Galectin-3, Galectin-1, EGFR still represented intra- and inter-cell line heterogeneity on A549, H1975 and H1650 cells. These markers were also investigated and showed differential expression in the primary human adenocarcinoma tissue compared to non-involved area. The qRT-PCR and SCMC revealed that hypoxic microenvironment and altered metabolism influence intratumor heterogeneity. Intra- and inter-cell line, moreover intratumor heterogeneity have been demonstrated by viSNE (visualization of stochastic neighbor embedding) high-dimensional and FlowSOM analysis also.

These results suggest that NSCLC cell lines bear intrinsic clonal heterogeneity which does not rely on the different phases of the cell cycle rather it depends on hypoxia and altered metabolism. Additionally, the intratumor heterogeneity of human primary adenocarcinoma has been revealed by single cell mass cytometry.

## Crosstalk of human bone marrow derived plasma cells and mesenchymal stromal cells in vitro

**Sarah Gräßle[1], Axel Ronald Schulz[1], Sabine Baumgart[1], Antonia Niedobitek[1], Stefanie Hahne[1], Eva Alina Holzhäuser[1], Pawel Durek[1], Tilo Dehne[2], Henrik Mei[1]**

[1]*Deutsches Rheuma-Forschungszentrum (DRFZ), Berlin, a Leibniz-Institute, Germany;* [2]*Charité Universitätsklinikum Berlin*

Long-lived plasma cell (PC) surviving in specialized niches within the human bone marrow maintains long-term humoral immunity by continuous secretion of specific antibodies. It is strongly suggested, that bone marrow (BM) stromal cells (SC) are an important part of these niches. While SC effects on PC are evident, the possibility that PC may act on SC and thus take part in shaping their own niche has been rarely addressed. We here analyzed the mutual interplay between primary human bone marrow PC and stromal cells grown and expanded from primary human BM tissue as a model for stromal cells.

We first investigated whether SC cultures are able to support PC survival in vitro. After 5 days of culture, 36,5% of PC survived in the presence of SC, 37,7% with SC supernatant, compared to 8,7% in control assays (3 experiments, functional PC detected by Elispot). SC cultures facilitated PC survival for at least 14 days in single samples.

After confirming that SC cultures recapitulate the PC-supporting role ascribed to primary SC, bulk transcriptome data of SC cultures and BMPC were mined for the expression of potentially interacting pair of molecules. Notably, we detected mRNA of several cytokines in human BMPC such as CCL3, BMP6 and VEGF. Flow cytometry was used for validation experiments on a single-cell, protein level. SC cultures were incubated with the same cytokines to address the functional consequences of PC-derived cytokines on SC, e.g. whether SC modulate the expression of cell adhesion and survival factors important for PC. As we found strong auto fluorescence in cultured SC limiting the utility of flow cytometry in these experiments, we established a 33 marker panel including CD90, CD105, CD44, CD29, APRIL, IL-6, and CXCL12, and others for mass cytometry. A 40-sample barcoding scheme using B2M antibodies facilitated joint analysis of differently stimulated SC and allowed spiking in PBMC serving as staining reference and acting against clogging during sample introduction. Preliminary analysis of one experiment suggests that factors secreted by PC modulate SC features. While analyses are ongoing and additional data is required, the available results indicate the presence of possible interactions beyond CXCR4/SDF-1, point towards mutual rather than unidirectional interactions, and highlight the utility of mass cytometry to analyze auto fluorescent stromal cells. Elucidating the PC interactions with their environment that facilitate their long-term survival will help understanding the organization of humoral memory and may have significant implications for the treatment of antibody mediated diseases.

## Dissociation and enrichment strategies for human thymic tissue in studies on antigen-presenting cells

**Marte Heimli[1], Hanne Sagsveen Hjorthaug[2], Siri Tennebø Flåm[2], Teodora Ribarska[2], Mario Saare[1], Benedicte Alexandra Lie[1]**

[1]*University of Oslo, Norway;* [2]*Oslo University Hospital, Norway*

Autoimmune diseases are characterized by the immune system raising aberrant responses towards the hosts own tissues. This instigates a need for identifying and eliminating potentially harmful, self-reactive immune cells during their development. For the T cells, development occurs in the thymus, and the progenitor cells are termed thymocytes. During the process of immunological education, T-cell receptors on the thymocytes are assessed for their affinities towards a wide variety of self-antigens. The self-antigens are presented in complex with MHC class II molecules on the surface of antigen-presenting cells, such as conventional and plasmacytoid dendritic cells, medullary and cortical thymic epithelial cells (mTECs and cTECs), and B cells. The mTECs are of special note, as they are involved in the ectopic expression of tissue-restricted antigens,

driven by the transcription factor Autoimmune Regulator (AIRE). This ensures that developing thymocytes also encounter these antigens, which normally would only be present in specific organs, within the thymic microenvironment. There is also emerging evidence of a large heterogeneity existing within the mTEC population.

Although immunological education is crucial in the prevention of autoimmune disease, little is yet known about the human thymus. Current knowledge is largely based on mice studies, despite the notion that there exist differences in the thymic structure and in marker genes expressed by different cell populations across the species.

We wish to perform a comprehensive mapping of the human thymus, and characterize cell populations based both on protein and RNA expression. The antigen-presenting cells are of particular interest. However, several of the applied techniques, such as mass cytometry and single-cell RNA sequencing, require the cells to be in suspension with a sufficient representation of relevant cell populations. Dissociation of thymic tissue for use in these studies represents a challenge due to the rarity and fragility of the antigen-presenting cells, the requirement for retaining epitopes that will be exploited for antibody staining, and the structural organization of the tissue, which includes highly keratinized bodies. To complement the techniques requiring cell suspensions, and in order to obtain data on spatial organization, we are also working on a protocol for antibody staining of thymic tissue slices using the Hyperion imaging system.

We have developed an optimized dissociation protocol for human thymus, based on testing of different enzymes, enzyme concentrations and incubation conditions. The resulting protocol allows for the survival of thymic populations of antigen-presenting cells, and the preservation of relevant marker proteins. This has been confirmed by use of flow cytometry and a mass cytometry panel, including, but not restricted to, the markers CD19, CD141, CD123, CD11b, HLA-DR and EpCAM. In addition, we have tested different enrichment protocols for antigen-presenting cells based on density gradient centrifugation. This has allowed a >10-fold enrichment of populations expressing CD19, CD141, CD123, and CD11b , respectively, resulting in populations that can be distinguished by use of mass cytometry. For the scarce population of EpCAM+ cells (≤ 0.5% among the enriched cell suspension), we have tested further enrichment based on depletion of CD45-expressing cells using antibody-coated magnetic beads. This strategy results in a high enrichment of EpCAM+ cells, but greatly reduces cell numbers, representing a challenge for mass cytometry staining protocols.

Our results suggests that choices regarding dissociation and enrichment protocols affect cell yield, cell survival, and frequencies of different cell populations, and thus may have implications for the results of mass cytometry studies.

## Application of mass cytometry for singe-cell nanotoxicology

**Sabine Baumgart[1], Ana Lopez-Serrano Oliver[2], Axel Ronald Schulz[1], Anette Peddinghaus[1], Doreen Wittke[3], Andrea Haase[3], Andreas Luch[3], Norbert Jakubowski[2,4], Henrik E. Mei[1], Andreas Grützkau[1]**

*[1]Deutsches Rheuma-Forschungszentrum (DRFZ), Berlin, a Leibniz-Institute, Germany; [2]Bundesanstalt für Materialforschung und -prüfung Berlin, Germany; [3]Bundesinstitut für Risikobewertung Berlin, Germany; [4]Spetec GmbH, Erding, Germany*

In the mass cytometry community, nanoparticles (NPs) are mainly considered as high-sensitivity mass tags for detecting low-abundance cell-surface markers. Since not only single cells but also metallic NPs with an atomic mass larger than 89 amu can be detected by mass cytometry, the technology suggests itself as a tool to study the impact of cell-NP interactions. Over the last years, NPs have increasingly penetrated the consumer marketplace including applications in medicine as e.g. contrast agents. Therefore, methodologies are needed that inform about the

safety vs potential hazards to human and environmental health.

To address the applicability of mass cytometry for studying the interaction between cells and NPs on a single-cell level, we here studied the uptake of silver nanoparticles (AgNPs) by individual THP-1 macrophages by mass cytometry. We further established a new calibration approach based on AgNPs suspension rather than ionic silver standard solutions for quantifying the amount of AgNPs associated to cells, serving as a valuable readout for cell-NP interactions in addition to multi-variate phenotypic information of individual cells.

Using differentiated THP-1 cells, we monitored and quantified the uptake of 50 nm citrate-coated AgNPs in a time and dose-dependent manner. After exposure of differentiated THP-1 cells to AgNPs at concentrations of 0.1 and 1.0 mg Ag/L for 4 or 24 h, 7 to 120 AgNPs per cell (2 to 89 fg Ag/cell) were determined. The results were validated by mass cytometric analysis of digested cells similar to conventional inductively coupled plasma mass spectrometry.

Our data show that based on the high sensitivity and single cell resolution of mass cytometry, even low amounts of cell-associated NP can be reliably quantified. This work highlights the utility of mass cytometry for nanotoxicology studies at the single-cell level.

## Patients suffering from immune-checkpoint inhibitor induced neurological adverse events show distinct patterns of immune activation

**Samuel Knauss[1,2], Axel Schulz[3], Lorena Ginesta Roque[1], Petra Hühnchen[1,2,4], Christian Schinke[1,2], Lucie Heinzerling[5], Henrik Mei[3], Wolfgang Böhmerle[1,2,4], Matthias Endres[1,2,4,6,7]**

*[1]Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Klinik und Hochschulambulanz für Neurologie, 10117 Berlin, Germany; [2]Berlin Institute of Health (BIH), Anna-Louisa-Karsch Straße 2, 10178 Berlin, Germany; [3]Mass Cytometry Lab, German Rheumatism Research Center (DRFZ), A Leibniz Institute, Berlin, Germany; [4]Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, NeuroCure Cluster of Excellence, 10117 Berlin, Germany; [5]Department of Dermatology, Universitätsklinikum Erlangen and Friedrich-Alexander-Universität (FAU) Erlangen-Nürnberg, Erlangen, Germany; [6]Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Center for Stroke Research Berlin, 10117 Berlin, Germany; [7]German Center for Neurodegenerative Diseases (DZNE), Berlin, Germany and German Center for Cardiovascular Research (DZHK), partner site Berlin, Germany*

Objective: The objective of the present study was to investigate the immunophenotype of patients exhibiting neurological immune related adverse events after checkpoint inhibitor therapy. To this effect, we analysed peripheral blood monocytic cells (PBMC) from patients receiving checkpoint inhibition with and without immune related adverse events.

Methods: We analysed PBMC from 18 patients receiving immune checkpoint blockade for treatment of malignant melanoma. Nine patients developed immune related adverse reactions after checkpoint inhibitor treatment. Eight Patients who did not develop immune related adverse reactions 6 months after first treatment served as controls. PBMC from control patients were analysed before treatment initiation (V0) and 6-12 weeks after first treatment (V1). We employed a previously tested CyTOF panel for immunophenotyping with a focus on T cell phenotyping. To calculate differences in cell composition we

used group comparisons in the FlowSOM framework.

Results: Comparing immune cell composition of control patients before and after treatment commencement revealed a distinct reaction of T cell subsets including CD4+ Cd27- CD28- and CD8+ Tbet-, CRCX3- TIGIT- cell populations. More importantly patients developing immune related adverse reactions after checkpoint inhibitor treatment exhibited a distinct expansion in a PD-1+, CD28/CD27+ CTLA4+ Tbet- and a CD4/CD8 double negative T cell subsets. We also observed a relative reduction in peripheral dentritic cells and non-classical monocytes when comparing to checkpoint inhibitor treated patients without immune related adverse events.

Conclusion: We found first evidence of a distinct immunophenotype of patients developing immune related adverse events after checkpoint inhibitor treatment.

## How to cope with massive Flow Data: UMAP and modern machine learning algorithms for efficient analysis of cytometry data

**Maren Büttner, Thomas Hofer, Elfriede Nößner, Fabian Theis**

*Helmholtz Zentrum München, Germany*

The analysis of flow cytometry data is usually approached by sophisticated gating strategies. However, setting gates is a manual process, which varies across persons, does not scale to high number of markers and hinders the discovery of new cell populations.

To overcome these limitations, we extended the single-cell RNA-sequencing analysis pipeline in Python (scanpy) to the flow cytometry data type to perform all analysis steps in an integrated framework. In particular, we present a machine learning approach to identify cell populations in flow cytometry data via clustering by community detection algorithms on all markers in a single step. By using UMAP for data visualization in 2D or 3D, our approach scales well with the number of parameters and the number of observations. Furthermore, all clusters can be annotated and visualized in 2D-scatter plots, similar to gating strategies. In addition, the cell type compositions per sample are directly accessible.

We demonstrate applicability of our scanpy extension to human PBMC data. Here, we found that the gating strategy for dendritic cells is a heterogeneous population with several distinct sub cell types.

## Skin immune cell infiltration: multidimensional single cell mass cytometry and flow cytometry studies of acne inversa lesions

**Christos Nikolaou[1,2,3], Theresa-Charlotte Brembach[1,2,3], Georgios Kokolakis[1,2], Désirée Kunkel[3,4], Andreas Thiel[1,3], Robert Sabat[1,2,3], Kerstin Wolk[1,2,3]**

*[1]Charité - Universitätsmedizin, Germany; [2]Psoriasis Research and Treatment Center, Institute of Medical Immunology & Department of Dermatology, Venereology and Allergology; [3]BIH Center for Regenerative Therapies; [4]Flow & Mass Cytometry Core Facility, Charité - Universitätsmedizin Berlin, Germany*

Chronic immune-mediated skin disorders represent an important public health problem due to their high prevalence and the tremendous impact on patients' quality of life. Understanding of the underlying immune mechanisms requires accurate characterization of the nature and function of immune cell subsets populating the diseased skin of these patients. Due to the com-

plexity of the skin organ, however, skin immune cell characterization remains a challenge.

Our current study aims at the establishment of a respective methodical approach and its application to characterize the skin immune cell infiltrate of patients suffering from acne inversa, a poorly characterized disease with a high medical need. Lesions comprise inflamed nodules, abscesses, and pus-discharging fistulas, developing in axillary, inguinal, gluteal, and perianal sites and which are very painful for the patients.

For these analyses, we chose single cell mass cytometry (CyTOF) in combination with multi-color flow cytometry, using a large range of markers that allows immune phenotyping of both resident and non-resident immune cells. In contrast to multi-color flow cytometry, CyTOF has been scarcely used so far within the field of investigative dermatology. CyTOF technology allows the simultaneous assessment of more than 45 cellular markers thus allowing deciphering the immunological heterogeneity in the human skin. As the first critical point in this approach, we developed an experimental method comprising of mechanical and enzymatic digestion of human skin suited for downstream cytometric applications that enable accurate data acquisition by optimizing signal detection and epitope recovery while minimizing background noise and low cell yields. We developed a 40 markers CyTOF panel for human leukocyte subsets identification comprised of a range of diverse markers for tissue homing, cytotoxicity and effector functions of these cells. Notably, chemokine receptors such as CXCR3, CCR6 and CCR4 vital for phenotyping of immune subsets of our interest are highly sensitive to isolation and preservation methods. Our results show that digestion with Collagenase I or IV with Benzonase for 6 hours at 37 oC maintain epitope stability but also does not alter cell viability while obtaining adequate cell yields. In contrast, Dispase II, which is often used in skin digestion protocols, digests even vital immune markers such as CD4 and thus is not preferable for our applications and panel.

First analysis of AI skin-derived cells revealed that memory CD3+ TCRab+ T cells represent the dominant 60% population among single live CD45+ mononuclear immune cells. In addition, granulocytes, B cells, NK cells and monocytes are infiltrating immune subsets in AI samples. Further characterization of the T cell subsets revealed both CD4+ and CD8+ memory subsets as well as distinct subsets of CD103+ CD69+, CD103- CD69+ and CD103- CD69- among CD4+ and CD8+ are present in the AI tissue. Further characterization of the CD19+ B cell subsets revealed that the dominant populations in the lesional areas are class switched IgM- IgD- and IgM+ IgD- CD24- CD38-.

We developed an optimized protocol for downstream multidimensional single cell mass cytometry applications which is applicable for various human cutaneous diseases such as hidradenitis suppurativa, psoriasis vulgaris and atopic dermatitis. Optimization between cell yield and epitope recovery, should be highly taken into consideration depending on markers of interest prior to skin CyTOF experiments in order to better permit comparisons between different tissues, blood but also donors as well as distinct time points of biopsy collection. We aim to characterize the skin immune cell infiltrate of patients suffering from chronic skin inflammatory diseases such as acne inversa in order to decipher causes,effects and manifestations possibly leading to a successful medical treatment.

## Establishment of a T cell exhaustion-directed workflow using high dimensional data analysis

**Lea Seidel[1,3], Saskia Killmer[1], Henrike Salié[1], Bertram Bengsch[1,2]**

*1Department of Medicine II, Gastroenterology, Hepatology, Endocrinology, and Infectious Diseases, University Medical Center Freiburg, Faculty of Medicine, Germany.; 2Signalling Research Centres BIOSS and CIBSS, University of Freiburg.; 3Faculty of Biology, SGBM*

Exhausted T cells (Tex) with functional limitati-

ons expressing targetable immune checkpoint receptors accumulate in many chronic diseases with key roles in chronic infection and many cancers. Recent evidence points to numerous subpopulations of Tex that have different biological roles and opposing clinical implications. Importantly, reinvigoration of Tex has been highlighted as a major correlate of successful cancer immunotherapies. However, comprehensive assessment of Tex diversity in human disease is lacking. In previous work, precise characterization of Tex subsets required a high-parametric approach profiling >16 exhaustion markers simultaneously. Mass cytometry is therefore perfectly suited to determine the heterogeneity and subset distribution of Tex.

At the newly established Freiburg mass cytometry site, we develop an integrated exhaustion-directed workflow based on 47+ channel mass cytometry. An exhaustion-specific marker backbone is combined with additional phenotypic and functional exhaustion profiling as well as exhaustion-directed signaling panels focused on TCR and metabolic signaling. Integration of data from different panels is possible through shared scaffold parameters.

In the tumor context, combining the mass cytometry approach with IMC allows the localized and spatial contextualization the different Tex subsets. The establishment of an exhaustion-directed immuno-profiling panel for the assessment of the tumor microenvironment in hepatocellular carcinoma is currently ongoing. Bioinformatic analysis of the data will focus on the characterization of different exhaustion signatures and the cellular interactions between tumor and immune cells.

In sum, we are currently establishing an exhaustion directed mass cytometry workflow that aims to provide important information on the T cell exhaustion status crucial for understanding the role of Tex in chronic infections and cancer, with the perspective to help guide personalized immunotherapy decisions.

## Deep phenotyping of human bone marrow plasma cells via mass cytometry

**Eva Holzhäuser, Axel Ronald Schulz, Antonia Niedobitek, Sabine Baumgart, René Riedel, Sarah Grässle, Henrik Mei**

*Deutsches Rheuma-Forschungszentrum (DRFZ), Berlin, a Leibniz-Institute, Germany*

Plasma cells (PC) in the human bone marrow (BM) mediate long-term humoral immunity. Among BMPC, CD19+ and CD19- PC can be distinguished, which have been linked to different PC life spans. Previous data indicate the differential expression of e.g. cell adhesion receptors in BMPC subsets, including markers commonly absent from B lineage cells such as CD28, CD56, and CCR2. While this suggests the existence of a significant phenotypic diversity of PC beyond the classification according to CD19 expression, a systematic analysis of PC phenotypes found in the human bone marrow is lacking. Here, we explore the phenotypic diversity of human bone marrow PC by mass cytometry.

We set up a workflow and a 45-marker panel to study the interrelatedness of PC phenotypes with the expression of immunoglobulin isotypes and subclasses, pro-survival mediators such as Bcl-2, cell adhesion and chemokine sensing receptors (including CD38, CD319, CD56, CD45, CCR2, CD49f, CD9, and others). CD138+ PC were isolated by MACS from suspensions of primary human femoral head BM cells, and stained for cell-surface and intracellular markers, including transcription factors. PC were defined as live, nucleated, CD38high cells co-expressing CD319 and exactly one immunoglobulin isotype or subclass. At average, 80.000 PC were available for analysis. Initial data exploration was performed by manual gating and opt-SNE.

The overall data confirm the known expression profile of PC, including high levels of B2M, CD38, CD27, IRF4 and CD319, low levels of HLA-DR, pax5 and CD20, as well as differential ex-

pression of CD19. The vast majority of PC did not express the proliferation marker Ki-67, showing that the most PC are resting during steady state. Beyond stratification of PC according to CD19 expression, we detected differential expression of CD56, CD45, CD28, CD44, CD49f, CD95, CCR2 and CCR10 in BMPC. The expression of these markers was partially but not completely overlapping, thus establishing the presence of a large number of distinct PC phenotypes. In pursuit of the idea that certain PC phenotypes may be associated with the type of Ig produced, we exemplarily analyzed the Ig subclass composition in eight PC subsets defined by combinatorial gating according to CD19, CD45 and CD56 expression. Notably, non-canonical CD56+ and CD45- PC phenotypes were detectable across all donors analyzed, indicating that these phenotypes, commonly linked to neoplastic PC, contribute to the normal PC pool. In addition, we found that these phenotypes were strongly enriched within CD19- PC, and contained high frequencies of IgG-expressing PC. While CD19-/CD56+ PC were strongly dominated by IgG1+ PC, CD19-/CD56- PC showed higher frequencies of IgG2 as compared to their CD56+ counterparts. IgG3+ PC were most abundant in CD19+/CD45-/CD56+ PC. Interestingly, the same PC subsets also showed differential expression of various cell adhesion receptors, suggesting that different PC subsets may have the capacity to reside in different niches, and to differentially respond to signals from their environment.

This work establishes a workflow and mass cytometry panel to study human plasma cells. First results point towards an unexpected phenotypical diversity among plasma cells of the human bone marrow, illustrating that the regulation of PC might be more complex than previously assumed. Understanding the diversity and dynamics of BMPC may aid the development of new treatments of antibody-mediated diseases and vaccines.

## SINGLE CELL PROFILING OF SIGNALTRANSDUCTION PATHWAYS IN PEDIATRIC T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

**Daniela Kuzilkova**

*CLIP (Childhood Leukemia Investigation Prague), Czech Republic*

Acute leukemia is the most common malignancy of childhood. Most of pediatric acute leukemias are derived from B-cells, about 15% of pediatric leukemias are from T-cell origin and lead to T-cell acute lymphoblastic leukemia (T-ALL). Treatment efficacy of T-ALL patients is comparable to other acute leukemia patients, however about 30% of T-ALL patients relapse and their prognosis dramatically decrease. Therefore further investigation of the biology and treatment possibilities of T-ALL is urgently needed.

Using single-cell mass cytometry we developed panel of 39 metal-labeled monoclonal antibodies (moAbs) identifying T-ALL blasts and non-malignant T-cells. Using phospho-specific moAbs and moAbs targeting proliferation and apoptosis we detected signal transduction upon in vitro treatment of 17 diagnostic and 5 relapse T-ALL samples with IL-7 (Jak/STAT5 pathway activator), Ruxolitinib (inhibitor of Jak1 and Jak2 kinase), BEZ-235 (inhibitor of PI3K and mTOR), combination of Ruxolitinib and BEZ-235 and Pervanadate (inhibitor of tyrosine phosphatases).

First, we evaluated quality of the data using previously described targets of stimuli. E.g. pSTAT5 was phosphorylated upon IL-7 stimulation (Wilcoxon test, $p<0.05$). Although pAkt was not significantly affected upon BEZ-235 treatment as expected, another Akt down-stream target 4E-BP1 was dephosphorylated after BEZ-235 treatment ($p<0.01$). We detected strong phosphorylation of pLck in all T-ALL samples after treatment with Pervanadate. In general, in T-ALL blasts we observed a constitutive hyper activation of many down-stream targets (e.g. pErk1/2, p-p38 or pAkt) compared to residual non-malignant T-cells.

Next, we compared specific subgroups of T-ALL patients regarding their prognosis. pRb and Ki-67, both markers of proliferating cells, were overexpressed in subgroup of patients respon-

ding rapidly to therapy (prednisone good responders) compared to patients responding slowly to therapy (prednisone poor responders, $p<0.05$). Additionally, we observed enhanced pRb in patients with low and very low level of minimal residual disease (MRD) compared to patients with high MRD after 2 weeks of treatment.

We also compared patients (n=6) with mutation in tumor-supressor gene PTEN (the main negative regulator of PI3K/Akt/mTOR pathway) with patients without PTEN mutation (n=11). The PTEN mutation caused loss of PTEN expression in 5/6 PTEN mutated patients. In patient #10 (PTEN mut) a PTEN expressing subclone was detected. We also observed correlation between PTEN mutation and cMyc expression ($p<0.001$). Next, we detected increased expression of CD38 in T-ALL patients without PTEN mutation compared to T-ALL patients with PTEN mutation ($p<0.05$). Using PCA based on all measured markers we were able to distinguish between patients with and without PTEN mutation. Surprisingly, phospho-profile detected by 8 phospho-specific moAbs of the two subgroups did not differ in PCA view. Next we compared T-ALL patients with NUP214/ABL1 fusion (n=2) with T-ALL patients without NUP214/ABL1 fusion (n=15). NUP214/ABL was described to be present in about 6% of T-ALL patients, the fusion leads to hyperactivation of ABL1 signaling. In our cohort we detected higher pSTAT5 and pCREB in NUP214/ABL1 positive patients compared to NUP214/ABL1 negative patients ($p<0.05$). In the PCA view based on phospho-markers NUP214/ABL1 positive T-ALL patients were clearly separated from other T-ALL patients.

In summary, using mass cytometry we phenotypically and functionally characterized pediatric T-ALL samples. We detected constitutively active signal transduction pathways in T-ALL blasts compared to residual non-malignant T-cells. We also detected changes in protein expression and/or phosphorylation associated with specific genetic subgroups of T-ALL patients (e.g. T-ALL patient with PTEN mutation of NUP214/ABL1 fusion). Hereby we proved the feasibility of CyTOF-based single-cell profiling of signal transduction pathways in pediatric T-ALL samples.

# Friday, January 24th

## Session 5: Invited Talks II

Chair: Henrik Mei

## High-dimensional cytometry for immunophenotyping

**Burkhard Becher**

*University Zurich, Switzerland*

In the early days of flow cytometry, the use of two to three independent parameters to describe and characterize cellular populations was sufficient to estimate the relative proportions of the major leukocyte populations in the blood. The increasing number of parameters which can be measured simultaneously allow for the concomitant characterization of phenotypic and functional properties at the single cell level and led to major discoveries including new cell types. The emergence of cytometers capable of measuring more than 25 parameters simultaneously brings about a new area of cytometry, where intelligent algorithms and computer-aided analysis allow for a thus far unprecedented view onto the single cell proteome. I will discuss recent advances in single cell cytometry and how we apply this technology to immunophenotype patients with inflammatory diseasefor the identifica-

tion of disease-relevant signatures for diagnosis, patient stratification and therapy responses.

## Explorative visual analytics for large single-cell data

**Thomas Höllt**

*Leiden University Medical Center, The Netherlands*

Visual Analytics (VA) combines computer graphics, human computer interaction, and perception to study and develop techniques to understand data through visual representations with machine learning to facilitate human-driven, analytics-supported visual exploration of large, complex datasets.

Such techniques offer tremendous potential for explorative data analysis and hypothesis generation in many application domains. In recent years, my team and I have made contributions defining and applying VA techniques to single-cell analysis.

In this talk, I will present examples on how to use VA methodology to interactively explore large-scale single-cell mass-cytometry data to form initial overview and generate hypothesis on the data. I will present our ultra-fast GPU-based t-SNE, the hierarchical HSNE and how we built complete workflows based on these techniques in Cytosplore, an interactive analysis software for large scale mass cytometry data.

### Session 6: Workshop Data Analysis

Chair: Marie Urbicht

## A visual interrogation of dimension reduction tools for single-cell analysis

**Tyler Joseph Burns**

*Berlin, Germany*

Dimension reduction tools are widely used within mass cytometry analysis, with t-SNE and UMAP being the most popular. Evaluation metrics for their performance have been seen more recently in the field, but these have focused more on t-SNE and do not evaluate how performance varies within a given map. Here, I use neighborhood-based analysis to evaluate both global and local data preservation between high-dimensional and reduced-dimensional data for t-SNE and UMAP. I find that t-SNE outperforms UMAP with respect to small neighborhoods around a given cell, and UMAP outperforms t-SNE with respect to neighborhoods farthest away from a given cell. Furthermore, how well the neighborhoods are preserved varies across a given map in well-defined patterns. My results give intuition around how well these tools are working, and they have strong implications around whether and how a dimension reduction map should be gated and/or clustered directly if one chooses to do so.

# A third trimester multi-omic clock predicts the spontaneous onset of labor

**Ina A Stelzer[1], Sajjad Ghaemi[1], Xiaoyuan Han[1], Kazuo Ando[1], Laura Peterson[2], Kevin Contrepois[3], Edward Ganio[1], Amy Tsai[1], Eileen Tsai[1], Kristen Rumer[1], Natalie Stanley[1], Ramin Fallazadeh[1], Martin Becker[1], Anthony Culos[1], Dyani Gaudilliere[1], Ronald J Wong[2], Virginia Winn[4], Gary M Shaw[2], David K Stevenson[2], Michael Snyder[3], Martin Angst[1], Nima Aghaeepour[1], Brice Gaudilliere[1]**

*[1]Department of Anesthesiology, Perioperative and Pain Medicine, School of Medicine, Stanford University, Palo Alto, CA, USA; [2]Department of Pediatrics, School of Medicine, Stanford University, Palo Alto, CA, USA; [3]Department of Genetics, School of Medicine, Stanford University, Palo Alto, CA, USA; [4]Department of Obstetrics and Gynecology, School of Medicine, Stanford University, Palo Alto, CA, USA;*

Introduction: Pre-term birth is the most common cause of mortality in children under the age of five world-wide. However, our ability to predict the onset of labor in preterm – or healthy – pregnancies is limited. In a recent study of healthy pregnancies, we described the chronology of immunologic, proteomic, metabolomic and transcriptomic adaptations occurring in the maternal blood during pregnancy. Here, we performed a multi-omic profile of longitudinal blood samples, collected during the third trimester of pregnancy in women with spontaneous onset of labor, to identify the molecular and cellular events that precede the onset of labor.

Material and Methods: Peripheral blood and plasma samples were collected weekly between the 28th and 40th gestational week in n=53 women who subsequently went into labor spontaneously (Mean GA at labor onset: 39w1d ±1w5d). In aggregate, collected samples spanned the third trimester with daily resolution. Whole system peripheral immune responses were analyzed using a high-dimensional mass cytometry immunoassay. A multiplex, aptamer-based protein detection platform was used to determine plasma protein levels. Metabolic and lipidomic analytes were detected using high-throughput mass spectrometry.

Results: A Lasso regression model predicted the difference between the day of sampling and the onset of labor with high accuracy ($r = 0.8511$, $p<0.0001$) and identified a striking molecular shift occurring 24 days before the onset of labor. The most informative features of the multi-variate model included the STAT1 signaling responses in CD56loCD16+NK cells, IL-33-receptor IL1R4, inflammation marker Cystatin C, and angiogenic Angiopoietin-2, anti-coagulant Antithrombin III and Progesterone.

Conclusion: The multi-omic assessment of maternal blood across different biological systems revealed a multivariate model of cellular and molecular events that accurately predicted the spontaneous onset of labor in healthy pregnancies. The results provide the foundation for testing the generalizability of the model for the prediction of preterm labor and identifying divergent mechanisms that drive the premature onset of labor.

Chair: Désirée Kunkel

## How many roads must a biomarker walk down, before you call it a BIOMARKER? Hope and Hype for new cellular biomarkers in rheumatology

**Andreas Grützkau**

*Deutsches Rheuma-Forschungszentrum (DRFZ), Berlin, a Leibniz-Institute, Germany*

Mass cytometry raised new hopes for the discovery of cell-based biomarkers that would set positive impulses in stratified medicine of chronic inflammatory rheumatic diseases. Here we will give a short overview about the destiny of cellular biomarkers identified since the beginning of the new millennium in rheumatology, such as plasmablasts/plasmacells, NK cell-, granulocyte- and monocyte-subsets. But most of these biomarkers have failed to get translated as diagnostic standards in the clinical routine, what will be discussed in light of new data generated by mass cytometry profiling of blood- and urinary leukocytes in systemic lupus erythemtosus (SLE). These studies revealed activated T cells and macrophage populations as potential non-invasive biomarkers detectable in urine of lupus nephritis (LN) patients. Since a sustained type I IFN response is a hallmark in lupus pathology, blockade of the JAK/STAT pathway is a promising new therapeutic option. In a proof-of-concept study we have combined in-depth phenotyping of blood leukocytes with cell type-specific intracellular signal transducer phosphorylation analyses to monitor treatment responses to the JAK-inhibitor Baricitinib. SLE patients showed a significant decrease in disease activity, which was paralleled by decreased levels of pSTAT1 phosphorylation in monocytes, CD4+ and CD8+ T cells.

Despite rather poor results in cellular biomarker discovery so far multidimensionality of mass cytometry raises hopes for the discovery of new candidates that will add spice to the idea of companion diagnostics as keys to personalized medicine.

## Modelling cell-type specific signalling networks by mass cytometry in colorectal cancer organoids

**Thomas Sell**

*Charité Berlin, Germany*

Colorectal cancers are driven by well-defined mutations, deregulating the PI3K, MAPK, TP53, and TGF-beta signalling pathways. These pathways are key for maintaining the stem cell niche, they regulate differentiation, and interact in complex ways, forming a deeply interconnected network. Because of this complexity it is non-trivial to estimate the effects of mutations in single network nodes on the entire network. To study this oncogenic signalling, we used 3D organoid models, both patient-derived and derived from transgenic mice, and interrogated them on the single cell level. We established a mass cytometry antibody panel, tailored to measure abundances of key signalling molecules, multiple cell type-specific markers, and several cell phenotype markers. We perturbed the oncogenic signalling network in various organoid lines, using a panel of therapeutically relevant substances. This high-dimensional, high-resolution approach enabled us to monitor dozens of network nodes in single cells, while simultaneously having information about cell differentiation states. We then used the perturbation data for quantitative network modelling, to identify changes in the network‘s wiring. The combination of the mass cytometry technique with mathematical modelling allowed us to pinpoint changes in signalling caused by cancer-related mutations and to identify differences in the signal transduction network‘s wiring between cell differentiation states, such as cell type-dependent differences in RAS to ERK signal transduction. We envisage

that our approach allows us to quantitatively define cell signalling networks and identify oncogene-dependent vulnerabilities in heterogeneous cancer cell populations, leading to the potential development of new treatment options.

## Characterisation of granzyme b positive myeloid cells with immunotherapeutic potential for colorectal cancer patients using imaging mass cytometry.

**Marieke Ijsselsteijn[1], Antonis Somarakis[2], Ruud van der Breggen[1], Frits Koning[3], Noel de Miranda[1]**

*[1]Department of pathology, Leiden university medical centre, The Netherlands; [2]Department of radiology, Leiden university medical centre, The Netherlands; [3]Department of immunohematology and blood transfusion, Leiden university medical centre, The Netherlands*

Large advancements have been made in cancer immunotherapy with promising results in the treatment of melanoma and NSCLC. Despite the relatively high mutation burden, colorectal cancers (CRC) often do not respond to immunotherapy, highlighting the need for deep characterisation of the tumour immune microenvironment in these tumours. This can be achieved through imaging mass cytometry as it combines the detection of a plethora of markers with spatial information. We developed a 40-marker panel for imaging mass cytometry on FFPE tissues which combines immune cell, cell state and tissue specific markers for the study of the CRC immune microenvironment. Furthermore, we developed an optimised workflow for maximum antibody performance by separating antibodies in two distinct incubation steps. Using this methodology, we characterised the previously described Granzyme B+ CD3- cell population in CRC and show that this population expresses CD11b, CD15 and CD45ro but does not express any major T-cell, B-cell or macrophage markers. The expression of proteins involved in cell killing prompted us to further investigate the localisation of the granzyme B myeloid cells and their interaction with the tumour. The complex multi-marker images were normalised through thresholding and binarisation on pixel level, followed by phenotype identification and interaction analyses using in house developed methodologies such as CytoSplore and ImaCyte. The interaction analysis highlighted that Granzyme B myeloid cells are associated with Ki67- tumour cells, indicating a lack of proliferation. This suggests that granzyme B myeloid cells indeed interact with the tumour and highlights their therapeutic potential for the treatment of CRC patients. Furthermore, the described 40 marker FFPE imaging mass cytometry panel is an excellent immune monitoring tool that can be readily applied in the context of cancer research.

## Session 8: Fluidigm Session

Chair: Henrik Mei

## Invited talk sponsored by Fluidigm: Systems omics for exploring melanoma subtypes

**Marie-Laure Yaspo, Berlin**

# Adressbook - 3rd German Mass Cytometry User Forum

| Name | Firstname | Institute | City | Country | Email |
|---|---|---|---|---|---|
| Adams | Robert | Biolegend | Koblenz | DE | robertadams@biolegend.com |
| Al-Maari | Mona | Miltenyi | Bergisch-Gladbach | DE | monaa@miltenyibiotec.de |
| Alföldi | Róbert | Astridbio Technologies Ltd. | Szeged | HU | r.alfoldi@astridbio.com |
| Andreas | Nico | Uniklinikum Jena | Jena | DE | nico.andreas@med.uni-jena.de |
| Aravamudhan | Sriram | Cell Signaling Technology | Leiden | NL | sriram.aravamudhan@cellsignal.com |
| Bakshi | Mayur | Fluidigm | | NL | mayur.bakshi@fluidigm.com |
| Balog | József Ágoston | Biological Research Centre | Szeged | HU | balog.jozsef@brc.hu |
| Baumgart | Sabine | DRFZ Berlin | Berlin | DE | sabine.baumgart@drfz.de |
| Becher | Burkhard | University Zurich | Zürich | CH | becher@immunology.uzh.ch |
| Bengsch | Bertram | University Medical Center Freiburg, Faculty of Medicine | Freiburg | DE | Bertram.Bengsch@uniklinik-freiburg.de |
| Bockhorn | Vera | DRFZ Berlin | Berlin | DE | vera.bockhorn@drfz.de |
| Böttcher | Chotima | Charité Universitätsmedizin Berlin | Berlin | DE | chotima.boettcher@charite.de |
| Braun | Michael | Beckman Coulter GmbH | Krefeld | DE | mbraun@beckman.com |
| Braun | Julian | Charité Universitätsmedizin Berlin | Berlin | DE | julian.braun@charite.de |
| Buchner | Maike | TUM | Munich | DE | maike.buchner@tum.de |
| Burns | Tyler Joseph | | Berlin | DE | tjburns@alumni.stanford.edu |
| Büttner | Maren | Helmholtz Zentrum München | Neuherberg | DE | maren.buettner@helmholtz-muenchen.de |
| Camponeschi | Alessandro | University of Gothenburg | Gothenburg | SE | Alessandro.camponeschi@gu.se |
| Chiarolla | Cristina Maria | University of Würzburg | Würzburg | DE | cristina.chiarolla@uni-wuerzburg.de |
| Cosma | Antonio | LIH | Esch-Sur-Alzette | LU | Antonio.Cosma@lih.lu |
| Dasenbrock | Heike | Fluidigm | | NL | heike.dasenbrock@fluidigm.com |
| Dietz | Sevina Patricia | Center for Regenerative Therapies TU Dresden | Dresden | DE | sevina.dietz@tu-dresden.de |
| Fernández Zapata | M. Camila | Charité Universitätsmedizin Berlin | Berlin | DE | maria.fernandez@charite.de |
| Fordoxel | Florent | Expedeon | Cambridge | UK | florent.fordoxel@expedeon |
| Gärtner | Fabian | Forschung Chirurgie 1 (AG Knippschild) | Ulm | DE | fabiangaertner@hotmail.de |

| Name | Firstname | Institute | City | Country | Email |
|---|---|---|---|---|---|
| Gätjen | Marcel | Becton Dickinson GmbH | | DE | marcel.gaetjen@bd.com |
| Giannoulis | Angeliki | Weizmann Institute of Science | Rehovot | IL | angeliki.giannoulis@weizmann.ac.il |
| Glauben | Rainer | Charité Universitätsmedizin Berlin | Berlin | DE | rainer.glauben@charite.de |
| Globig | Anna-Maria | Universitätsklinikum Freiburg | Freiburg | DE | anna-maria.globig@uniklinik-freiburg.de |
| Gräßle | Sarah | DRFZ Berlin | Berlin | DE | sarah.graessle@drfz.de |
| Grützkau | Andreas | DRFZ Berlin | Berlin | DE | gruetzkau@drfz.de |
| Hameetman | Marjolijn | LUMC | Leiden | NL | m.hameetman@lumc.nl |
| Hasheminasab | Sayedmohammad | Charité Universitätsmedizin Berlin | Berlin | De | mohammad.hasheminasab@charite.de |
| Heimli | Marte | University of Oslo | Oslo | NO | marte.heimli@medisin.uio.no |
| Hemelen | Dries van | Fluidigm | | NL | Dries.VanHemelen@fluidigm.com |
| Hipp | Anna | Universitätsklinikum Freiburg | Freiburg | DE | anna.hipp@uniklinik-freiburg.de |
| Hirscher | Jacqueline | DRFZ Berlin | Berlin | DE | hirscher@drfz.de |
| Hirseland | Heike | DRFZ Berlin | Berlin | DE | hirseland@drfz.de |
| Hjorthaug | Hanne Sagsveeen | Oslo University Hospital | Oslo | NO | uxhjhb@ous-hf.no |
| Hoffmann | Ute | DRFZ Berlin | Berlin | DE | hoffmann@drfz.de |
| Höllt | Thomas | Leiden University Medical Center | Leiden | NL | T.Hollt-1@tudelft.nl |
| Hölsken | Oliver | Charité Universitätsmedizin Berlin | Berlin | DE | oliver.hoelsken@charite.de |
| Holzhäuser | Eva | DRFZ Berlin | Berlin | DE | eva.holzhaeuser@posteo.de |
| Hönig | Manfred | University Medical Center Ulm | Ulm | DE | manfred.hoenig@uniklinik-ulm.de |
| Horn | Veronika | Charité Universitätsmedizin Berlin | Berlin | DE | veronika.horn@charite.de |
| Ijsselsteijn | Marieke Erica | Leiden University Medical Center | Leiden | NL | m.e.ijsselsteijn@Lumc.nl |
| Kalina | Tomas | Charles University, 2nd Faculty of Medicine | Praha | CZ | tomas.kalina@lfmotol.cuni.cz |
| Kamradt | Thomas | Universitätsklinikum Jena | Jena | DE | thomas.kamradt@med.uni-jena.de |
| Keppler | Selina Jessica | Rechts der Isar - TUM | München | DE | selina.keppler@tum.de |
| Keye | Jacqueline | Charité Universitätsmedizin Berlin BIH | Berlin | DE | jacqueline.keye@charite.de |
| Killmer | Saskia | University Hospital Freiburg | Freiburg | DE | saskia.killmer@uniklinik-freiburg.de |
| Konig | Marion | LUMC | Leiden | NL | m.h.koenig@lumc.nl |
| König | David | DRFZ Berlin | Berlin | DE | david.koenig@drfz.de |

| Name | Firstname | Institute | City | Country | Email |
|---|---|---|---|---|---|
| Koppejan | Hester | Leiden University Medical Center | Waddinxveen | NL | h.j.koppejan@lumc.nl |
| Kühl | Anja Andrea | Charité-Universitäts-medizin Berlin | Berlin | DE | anja.kuehl@charite.de |
| Kunkel | Désirée | BIH Cytometry Core | Berlin | DE | desiree.kunkel@charite.de |
| Kuzilkova | Daniela | CLIP (Childhood Leukemia Investiga-tion Prague) | Prague | CZ | daniela.kuzilkova@lfmotol.cuni.cz |
| Laban | Sandra | LUMC | Leiden | NL | S.Laban@lumc.nl |
| Lehmann | Malte | Charité Universitäts-medizin Berlin | Berlin | DE | malte.lehmann@charite.de |
| Leipold | Michael | Stanford University School of Medicine | Stanford | US | mleipold@stanford.edu |
| Letizia | Marilena | Charité Universitäts-medizin Berlin | Berlin | DE | marilena.letizia@charite.de |
| Lüdiger | Stefanie | Biolegend | | | sluediger@biolegend.com |
| Mei | Henrik | DRFZ Berlin | Berlin | DE | mei@drfz.de |
| Midelet | Johanna | Expedeon | Cambridge | UK | johanna.midelet@expedeon.com |
| Mühle | Kerstin | Charité Universitäts-medizin Berlin | Berlin | DE | kerstin.muehle@charite.de |
| Neuperger | Patrícia | Biological Research Centre | Szeged | HU | neupergerpatri@gmail.com |
| Niedobitek | Antonia | DRFZ Berlin | Berlin | DE | antonia.niedobitek@drfz.de |
| Nikolaou | Christos | Charité Universitäts-medizin Berlin | Berlin | DE | christos.nikolaou@charite.de |
| Notø | Heidi Ødegaard | Oslo University Hos-pital | Oslo | NO | heinot@rr-research.no |
| Olsen | Lars Rønn | Technical University of Denmark | Kgs Lyngby | DK | lronn@dtu.dk |
| Paclik | Daniela | Charité Universitäts-medizin Berlin | Berlin | DE | daniela.paclik@charite.de |
| Rahimi | Habib | University Hospital Ulm | Ulm | DE | abdul-habib.rahimi@uni-ulm.de |
| Raifer | Hartmann | University of Marburg | Marburg | DE | raifer@staff.uni-marburg.de |
| Reinhardt | Julia | TU Dresden | Dresden | DE | julia.reinhardt@tu-dresden.de |
| Reudelsterz | Marc | Fluidigm | Berlin | DE | marc.reudelsterz@fluidigm.com |
| Riedel | René | DRFZ Berlin | Berlin | DE | riedel@drfz.de |
| Rieking | Thorsten | Omni-Life Science Gmbh & Co KG | Bremen | DE | tr@ols-bio |
| Rius Rigau | Aleix | Universitätsklinikum Erlangen | Erlangen | DE | AleixRius.Rigau@uk-erlangen.de |
| Rodríguez Sillke | Yasmina | Charité Universitäts-medizin Berlin | Berlin | DE | yasmina.rodriguez-sillke@charite.de |
| Romero Olmedo | Addi Josua | Philipps-Universität Marburg | Marburg | DE | addi.romero@staff.uni-marburg.de |

| Name | Firstname | Institute | City | Country | Email |
|---|---|---|---|---|---|
| Sai | Somesh | MDC-BIMSB | Berlin | DE | somesh.sai@mdc-berlin.de |
| Salame | Tomer Meir | Weizmann Institute of Science | Rehovot | IL | tomer-meir.salame@weizmann.ac.il |
| Salié | Henrike | University Medical Center Freiburg, Faculty of Medicine | Freiburg | DE | henrike.salie@uniklinik-freiburg.de |
| Sarkander | Jana | Biolegend | | DE | jsarkander@biolegend.com |
| Schmidt | Franziska | Charité Universitätsmedizin Berlin BIH | Berlin | DE | f.schmidt@charite.de |
| Schulz | Axel | DRFZ Berlin | Berlin | DE | axel.schulz@drfz.de |
| Seidel | Lea Malwa | Uniklinikum Freiburg | Freiburg | DE | lea.seidel@uniklinik-freiburg.de |
| Sell | Thomas | Charité Universitätsmedizin Berlin | Berlin | DE | thomas.sell@charite.de |
| Simpson | Simon | Fluidigm | | NL | simon.simpson@fluidigm.com |
| Sörensen | Till-Antoni | Charité Universitätsmedizin Berlin | Berlin | DE | till-antoni.soerensen@charite.de |
| Sos | Martin | University Cologne | Köln | DE | martin.sos@uni-koeln.de |
| Stadler | Margit | Cell Signaling Technology | | DE | margit.stadler@cellsignal.com |
| Stanislawiak | Silke | DRFZ Berlin | Berlin | DE | silke.stanislawiak@drfz.de |
| Stelzer | Ina | Stanford University | Stanford | US | istelzer@stanford.edu |
| Stöhr | Susanne | Becton Dickinson GmbH | Berlin | DE | susanne.stoehr@bd.com |
| Strehl | Cindy | Charité Universitätsmedizin Berlin | Berlin | DE | cindy.strehl@charite.de |
| Suwandi | Jessica | Leiden University Medical Center | Leiden | NL | j.s.suwandi@lumc.nl |
| Szebeni | Gabor J. | Biological Research Centre | Szeged | HU | szebenigabi@gmail.com |
| Ugursu | Bilge | Charité Universitätsmedizin Berlin | Berlin | DE | bilgenisan@gmail.com |
| Urbicht | Marie | DRFZ Berlin | Berlin | DE | marie.urbicht@drfz.de |
| Ursu | Simona-Felicia | Ulm University | Ulm | DE | simona.ursu@uni-ulm.de |
| Van Meijgaarden | Krista Elisabeth | Leiden University Medical Center | Leiden | NL | K.e.van_meijgaarden@lumc.nl |
| Warth | Sarah | Ulm University | Ulm | DE | sarah.warth@uni-ulm.de |
| Yaspo | Marie-Laure | Max Planck Institute for molecular Genetics | Berlin | DE | yaspo@molgen.mpg.de |