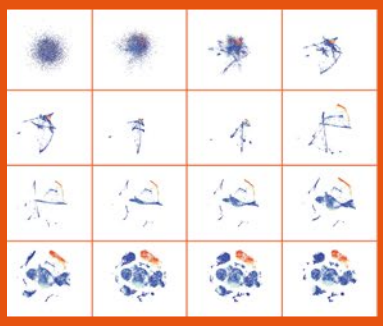

2nd German Mass Cytometry User Forum

January 24-25, 2019, Berlin

GerMaNet
German Mass Cytometry Network

Abstract book
January 24.-25, 2019

Venue:

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

We are moste grateful to our Sponsors and Exhibitors

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Dear friends,

After a successful and inspiring 1st Forum, we not only decided to continue the meeting series, but also to extend the the program to two entire days full of talks and workshops, and plenty of time for poster viewing and discussions.

I am very much looking forward to an interesting program around this exciting technology, which will feature internationally recognized speakers from the field of mass cytometry and bioinformatics. Workshops on mass cytometry basics & reagents, as well as 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 would like to thank the commercial exhibitors for their generous financial support to make this meeting possible.

I wish you all an exciting meeting and a pleasant stay in Berlin.

Yours,

Henrik Mei

Thursday, January 24th

8:30 Arrival and registration

9:30 Welcome (Henrk Mei)

Session 1

10:00 Elena Hsieh: Single-cell Systems Immunology: Applications in Primary Immunodeficiency

10:45 *Short Coffee Break*

11:00 Andrew Duckworth: Simultaneous profiling of antigen and RNA expression in individual cells using PLAYR and mass cytometry

11:45 Cara Wogsland: Systems immunology of murine tumors in the context of obesity

12:30 *Lunch Break*

Session 2

Presentation of selected abstracts (15 + 10 min)

14:00 Marilena Letizia: Mass cytometry profiling of adipose tissue immune cells in murine models of Inflammatory Bowel Disease

Sabine Baumgart: Exploring immune cell dynamics in inflammatory bowel diseases and their therapeutic modulation by TNF-blockers

Axel Schulz: Immune cell profiling of rheumatoid arthritis patients undergoing helminth therapy by mass cytometry

15:15 *Coffee break*

Session 3

16:00-18:00 **Workshop "Mass cytometry basics and reagents"**

Presentation of selected abstracts (15 + 10 min)

Eva-Maria Jacobsen: HLA-specific antibodies: a method for barcoding donor and recipient cells in patients with mixed chimerism after HLA-non-identical stem cell transplantation

Lisa Budzinski: Osmium-labeled Microspheres for Bead-based Assays in Mass Cytometry

Panel discussion (Moderator: Desiree Kunkel)

Poster presentation and get together

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

Friday, January 25th

Session 4

9:00 Bertram Bengsch: Deep immune profiling of exhausted T cells

9:45 Sofie Van Gassen: Discovering cell populations with FlowSOM clustering

10:30 *Coffee break*

Session 5

11:00 **Workshop "Data analysis"**

Manfred Claassen: (Un-)supervised learning of cell population structure from single-cell snapshot data

Presentation of selected abstracts (15 + 10 min)

Jan Verhoeff: INFERENCE, a comprehensive mass cytometry analysis pipeline

Panel discussion (Moderator: Bertram Bengsch)

13:00 *Lunch*

Session 6

Presentation of selected abstracts (15 + 10 min)

14:30 Kathrin Balz: RNA viruses induce heterologous immunity against environmental allergens

Marieke Ijsselsteijn: Next-gen immune profiling using imaging mass cytometry as a frame work for cancer immunotherapy

15:30 *Coffee break*

16:00 *Imaging Mass Cytometry supported by Fluidigm*
Jana Fischer: Investigating the Single-Cell Pathology Landscape of Breast Cancer using Imaging Mass Cytometry

17:00 *Wrap-up and farewell*

Impressions of the 1st GErman Mass Cytometry User Forum, 2018, Berlin, DRFZ

Venue of the 2nd German Mass Cytometry User Forum

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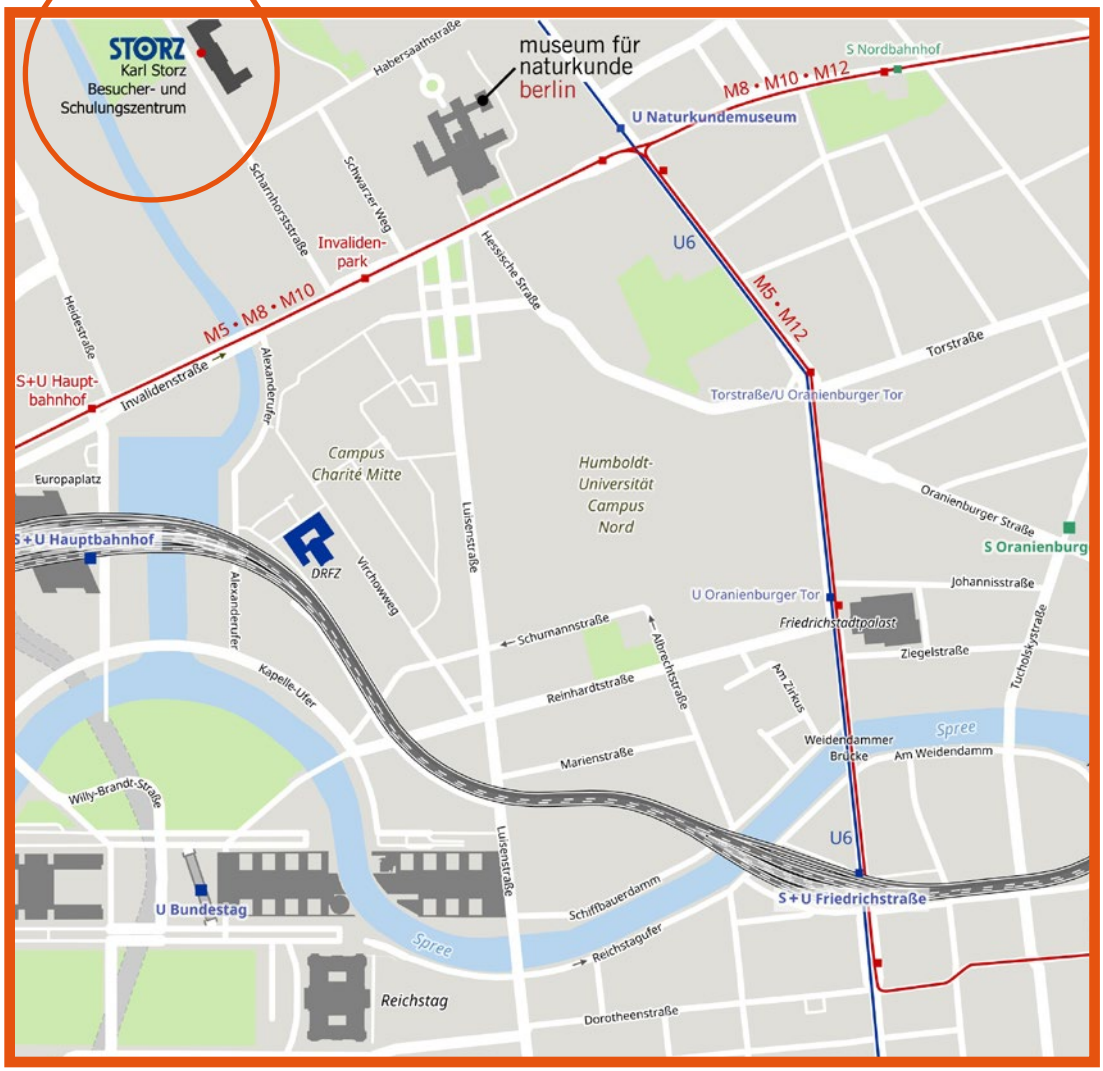

Distances
Berlin-Hauptbahnhof
(Central Station): 1 Kilometer
Tegel Airport: 9 Kilometer
Schönefeld Airport: 25 Kilometer

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 24th

Session 1

Single-cell Systems Immunology: Applications in Primary Immunodeficiency (PID)

Elena Hsieh

University of Colorado Denver, Children's Hospital Colorado, United States of America

Primary immunodeficiencies (PID) are a heterogeneous group of genetic disorders that cause altered composition and/or function of the immune system and result in immune dysregulation syndromes where autoimmunity is intertwined with microbial susceptibility. Even in the advent of whole exome sequencing (WES), the best approach for identifying specific immunopathology in individual with PID patients remains elusive. Challenging factors include i) the complexity of genetic variant identification and interpretation from WES, and ii) the imprecise downstream immune phenotypic and signaling ramifications of analyzed variants. Therefore, validation of these variants as disease-causing mutations still requires functional assays to explain patient-specific cellular and tissue pathophysiology, including the analysis of many immune parameters in many cells with single-cell resolution. We applied mass cytometry to understand the immunophenotypic and functional consequences of two novel PIDs, involving cytokine signaling (i.e. multiple pSTATs) and T/B cell receptor signaling networks (including proximal and distal read outs). We applied visNE and phenograph, to visualize overall immune cell subset population "hierarchy" changes; and DREVI/DREMI algorithm along with traditional heatmaps were used to understand signaling abnormalities. These studies demonstrated unique "signaling signatures" that underlie immune dysregulation mechanisms in these defects, and revealed novel biological insight regarding cytokine and TCR signal transduction and their effects on lymphocyte development.

Simultaneous profiling of antigen and RNA expression in individual cells using PLAYR and mass cytometry

Andrew Duckworth

University of Liverpool, United Kingdom

Proximity ligation assay for RNA (PLAYR) enables direct and highly multiplexed quantification of RNA in single cells by cytometry while maintaining the potential to decipher cellular identity in mixed populations through antibody staining of surface and internal antigen. We have established the use of PLAYR to measure RNA in our cells of interest (primary CLL cells and B cell lines) by both flow and mass cytometry. The signal intensity using these detection methods is comparable and is also highly concordant with those generated by qPCR. Increasing phenotyping potential to include RNA expression provides new horizons for mass cytometry.

Systems immunology of murine tumors in the context of obesity

Cara Wogsland

University of Bergen

Obesity is associated with increased cancer development, progression, and mortality in 13 cancer types including breast cancer and pancreatic cancer. Murine studies have found enhanced tumor growth and increased metastasis in obese mice compared to non-obese mice. Obese patients experience low levels of systemic inflammation, likely due to adipocyte necrosis in the expanding adipose tissue. It's unclear what the tumor immune environment looks like in obesity. Here, mass cytometry was used to characterize immune cell infiltrate in

tumors from obese and non-obese mice using syngeneic breast or pancreatic cancer models. Mice on either chow or high fat diet were injected orthotopically with cancer cells. Tumor growth was tracked overtime and tumors were collected and dissociated for mass cytometry analysis. A broadly focused immunophenotyping CyTOF panel of 37 antibodies was used to characterize the major immune cell types in murine tumors. The analysis pipeline included viSNE, SPADE, MEM, and CITRUS, with statistical analysis of clusters. We observed significant immune cell differences between breast cancer in the mammary fat pad in obese compared to non-obese mice. Interestingly, such difference was absent in pancreatic tumors – suggestive of tissue specificity of obesity-induced tumors.

Session 2

Mass cytometry profiling of adipose tissue immune cells in murine models of Inflammatory Bowel Disease.

Marilena Letizia[1], Yasmina Rodriguez Sillke[1], Franziska Schmidt[1], Claudia Günther[2], Désirée Kunkel[3], Rainer Glauben[1], Britta Siegmund[1], Carl Weidinger[1]

[1]Charité Universitätsmedizin Berlin, Germany;
[2]Universitätsklinikum Erlangen;
[3]Berlin-Brandenburg Center for Regenerative Therapies, Germany

Crohn's disease is characterized by epithelial barrier breaches and a subsequent translocation of bacteria from the intestinal lumen into the adjacent mesenteric fat, inducing fat hyperplasia as well as the recruitment of various immune cells. Nevertheless, the functional role of adipose tissue in intestinal auto-immunity is unknown and it remains elusive. Therefore, we compared the immune cell compartment of mesenteric fat, gonadal fat, mesenteric lymph nodes and intestinal lamina propria in mouse models of inflammatory bowel disease. Particularly, we focused on acute or chronic DSS-induced colitis as a model for colonic inflammation, whereas intestinal epithelial specific caspase-8 (Casp8ΔIEC) knockout mice have been used as a model for terminal ileitis, recapitulating features of Crohn´s disease.

To induce acute or chronic colitis, C57BL/6 mice were either fed 2.5% DSS in their drinking water for 5 days or received 4 cycles of 1.5% DSS for 7 days followed by 7 DSS-free days, respectively. Intestinal epithelial specific caspase-8 (Casp8ΔIEC) knockout mice were compared to wild type littermates. Immune cells were isolated from mesenteric fat, gonadal fat, mesenteric lymph nodes and intestinal lamina propria and subsequently analyzed by mass cytometry using a panel of 36 lineage and functional markers.

Our data provide for the first time a comprehensive, comparative immune cell characterization of lamina propria, mesenteric lymph nodes, mesenteric fat and gonadal fat in DSS-induced colitis or Casp8ΔIEC-induced ileitis. In all 3 models, immunosuppressive CD206+ macrophages were the most abundant myeloid cells found within adipose tissue. Interestingly, in acute DSS colitis mesenteric fat gained pro-inflammatory characteristics as TNFα production was induced in CD206+ macrophages, which could not be observed in chronic DSS induced colitis. In contrast, we observed that CD206+ macrophages infiltrating mesenteric fat of mice with ileitis displayed an up-regulation of anti-inflammatory markers such as CD38 and a reduction of TNFα production. Moreover, only the mesenteric fat of Casp8ΔIEC mice and not DSS colitis models showed infiltration of Ly6G+ neutrophils, while adipose tissue of all models showed an enrichment in innate lymphoid cells.

Our data suggest for the first time a dynamic immune-modulatory function of mesenteric fat in relation to location and development of intestinal inflammation driven by epithelial damage, highlighting a specific anti-inflammatory function of fat tissue upon transmural inflammation. Further functional assays have to be performed in order to assess a protective function of mesenteric wrapping fat in Crohn's disease.

Exploring immune cell dynamics in inflammatory bowel diseases and their therapeutic modulation by TNF-blockers

Sabine Baumgart[1], Marie Urbicht[1], Tyler Burns[1], Heike Hirseland[1], Anette Peddinghaus[1], Axel Schulz[1], Henrik E. Mei[1], Konrad Aden[2], Andreas Grützkau[1]

[1]DRFZ Berlin, a Leibniz-Institute, Germany; [2]Department of Internal Medicine I, University Hospital Schleswig-Holstein, Kiel, Germany; Institute of Clinical Molecular Biology, University of Kiel, Germany

OBJECTIVE: Crohn's disease (CD) is a chronic inflammatory disease affecting the entire gastrointestinal tract, whereby the inflammation often occurs in patches and spreads into deep layers of the intestinal tissue. Unlike CD, in ulcerative colitis (UC) the innermost mucosal lining of the colon or rectum becomes chronically inflamed. The hallmark of active IBD is an infiltration of innate and adaptive immune cells into the intestinal mucosa. To characterize IBD-related immune cell responses, we analyzed peripheral blood cells from active and inactive CD and UC patients at base line before TNF-¤ therapy and compared them to a group of sex- and age-matched healthy controls (HD) by mass cytometry.

METHOD: We applied a 43 parameter immunophenotyping panel, which was established to be compatible with Smart Tube fixed blood samples. In total, 160 samples from 37 IBD patients (UC: n=17; CD: n=20) and 30 sex- and age-matched healthy controls were monitored longitudinally at base line, 1d, 2, 6 and 16 weeks after starting treatment with the TNF-¤ blocker Infliximab. Samples were barcoded and analyzed on a Helios mass cytometer elios). Manually debarcoded data were analyzed using a bioinformatic pipeline based on the FlowSOM algorithm. Cells were randomly subsampled down to 50,000 cells forming 50 clusters in total.

RESULTS: Our mass cytometry pipeline allowed robust analysis of stabilized and frozen whole blood samples. The FlowSOM clustering algorithm identified statistically differences within active CD and UC patients compared to HD. In both diseases, frequencies of peripheral NK cells, B cells, DC's, monocytes, eosinophils, and TCRgd CD4-CD8- T cells were significantly decreased in comparison to the HD group. So, IBD-common and disease-specific cellular changes were detectable. Cell clusters identified were analyzed longitudinally in response to anti-TNF-¤ treatment. Here non-classical monocyte, dendritic cell and NK cell subsets, which were found to be decreased at baseline when compared to healthy controls, showed increased frequencies over time in clinical responders.

SUMMARY & CONCLUSION: Our analysis revealed commonly regulated immunophenotypic changes in CD and UC, which reflect rather unspecific chronic inflammatory responses. In addition, disease-specific differences were observed. Most interestingly, immune cell dynamics of predominantly innate phenotypes could be observed that correlated with the clinical response to infliximab. In conclusion, our study demonstrated the power of mass cytometry to identify promising immune cell signatures of therapy responses in peripheral blood that are of interest as diagnostic biomarkers for a disease and therapy stratification in IBD and other chronic inflammatory diseases.

Immune cell profiling of rheumatoid arthritis patients undergoing helminth therapy by mass cytometry

Axel Schulz[1], Silke Stanislawiak[1], Sabine Baumgart[1], Tyler Burns[1], Julia Patermann[2], Sandra Burger[2], Andreas Grützkau[1], Henrik E. Mei[1]

[1]DRFZ, Germany;
[2] Abt. Innere Medizin, Rheumatologie und Klinische Immunologie, Immanuel Krankenhaus Berlin, Germany

Based on the hygiene hypothesis, the treatment of rheumatoid arthritis (RA) by immune modulation induced by infection with eggs of the intestinal helminth Trichuris suis (TSO) promises amelioration of the disease, associated with fewer side effects as compared to currently approved therapies. In order to gain insight in the mechanism of action of TSO therapy and to facilitate precision medicine in the treatment of RA, we studied the composition and activation state of PBMC of RA patients undergoing targeted and timely controlled TSO treatment in a placebo-controlled trial by mass cytometry. We developed a mass cytometric antibody panel comprising 44 markers for complex immune profiling focused on T and B cell subsets and their activation status. A high degree of assay standardization was achieved by novel beta-2 microglobulin-based livecell barcoding in conjunction with a new preservation method for metal-labeled antibody cocktails.

The comparison of 31 age- and gender-matched healthy controls with 36 RA patients prior to TSO therapy revealed numerous RA-related phenotypical disturbances in the T cell, B cell and monocyte lineages. RA patients showed diminished frequencies of MAIT cells, significantly lower frequencies of IgG- and IgM- memory B cells and plasmablasts, while a subset of CD14hi CD16lo monocytes was increased. Furthermore, we identified CXCR3 to be expressed at significantly lower levels in RA patients on different T, B and NK cell subsets. After treatment with TSO, RA patients exhibited increased levels of CD23+ naive B cells and γδ T cells, but only transiently by week 13, when compared to placebo-treated patients. Ongoing work aims at further delineating trajectories of immune cell subsets over the course of the treatment and correlating them with the response to TSO and the clinical improvement of RA in the patients.

This study demonstrates the utility of massively high-parametric immune profiling by mass cytometry in chronic inflammatory conditions to identify immune cell aberrations for further consideration in immunopathogenesis research and therapy.

Session 3

Workshop 1: Mass cytometry basics and reagents

HLA-specific antibodies: a method for barcoding donor and recipient cells in patients with mixed chimerism after HLA-non-identical stem cell transplantation

Eva-Maria Jacobsen, Ansgar Schulz, Manfred Hönig

University Medical Center Ulm, Germany

Staining with allele-specific antibodies targeting HLA-molecules can distinguish donor and recipient cells in patients after HLA-haploidentical stem cell transplantation in flow cytometry. In patients with primary immunodeficiency, this tool allows to study the development and function of autologous cells in patients with

mixed chimerism. We demonstrate the developmental arrest of B cells originating from patients with B-cell positive SCID due to mutations in IL2RG or JAK3 in the presence of a functional donor derived T-cell system. The pool of autologous CD27-positive B cells contains merely MZ-like cells, class switched memory B cells are absent. We are planning to extend our protocols to mass cytometry and to combine barcoding of donor and recipient cells with intracellular stainings to obtain functional data after stimulation with specific and non-specific stimulants.

Beyond this, there are multiple other potential options to use this technology for immunological studies in patients after HLA-non-identical hematopoetic stem cell or solid organ transplantation with mixed chimerism.

Osmium-labeled Microspheres for Bead-based Assays in Mass Cytometry

Lisa Budzinski, Axel R Schulz, Sabine Baumgart, Heike Hirseland, Henrik E Mei

German Rheumatism Research Center - A Leibniz institute, Germany;

Polystyrene microspheres are broadly applied in flow cytometry for instrument setup and monitoring instrument stability, for assessing fluorescent spillover and in various cytometric assays e.g. for absolute quantification of cellular receptors and multi-analyte profiling. The implementation of bead-based assays in mass cytometry for the same purposes is strongly desired but hampered by the lack of functionalized beads associated with sufficient amounts of heavy metal allowing for the unequivocal detection of these by the mass cytometer.

We here introduce osmium tetroxide labeling for polystyrene microspheres as a simple, quick, and universal approach to produce various kinds of functionalized beads applicable in mass cytometry. Osmium detection does not interfere with any existing reagents routinely used in mass cytometric assays, and osmium labeling of various commercially available antibody capture beads resulted in stably and uniformly labeled beads, while retaining their antibody-capture functionality. Osmium-labeled antibody capture beads retained both their functionality and osmium signal for at least two weeks when stored at - 80 °C.

We show that osmium-labeled antibody capture beads can be employed for uncompromised characterization of metal-antibody conjugates, and for signal spillover assessment in complex mass cytometric data sets resulting from e.g. minor isotopic impurities of metal labels. Furthermore, osmium-labeled beads facilitate the absolute quantification of cell-surface receptors in mass cytometry.

Osmium labeling of polystyrene beads permits robust implementation of beads-based assays in mass cytometry, broadening the applicability of mass cytometry in biomedical and basic research, and increasing the quality and quantity of information retrievable in mass cytometric studies.

Friday, 25/Jan/2019

Session 4

Deep immune profiling of exhausted T cells

Bertram Bengsch

Universitätsklinikum Freiburg, Germany;

Exhausted CD8 T cells (TEX) are a distinct T cell lineage and immunotherapy targets in chronic infection and cancer but poorly defined in human diseases. We therefore developed a transcriptomic- and epigenetic-guided mass cytometry approach based on exhaustion-specific genes that identified at least 9 distinct TEX clusters in HIV and lung cancer by phenotypic, functional and transcription factor and inhibitory receptor co-expression patterns. An exhaustion severity metric was integrated with high-dimensional phenotypes to define TEX clusters that were: shared across chronic infection and cancer or enriched in either disease; linked to disease severity; and changed with HIV therapy. These data also identified combinatorial patterns of immunotherapy targets on different TEX clusters. Profiling TEX heterogeneity has implications for immune-monitoring and immunomodulation in chronic infections, autoimmunity and cancer.

Discovering cell populations with FlowSOM clustering

Sofie Van Gassen

Ghent University, Belgium

In the last decade, the number of proteins that can be measured simultaneously with cytometry techniques has increased tremendously. For mass cytometry especially, the traditional analysis method of repetitively selecting cell populations of interest in 2D scatter plots falls short. Not all possible 2D combinations can be examined and analysis results get biased towards the expected populations. Many cells are `gated out‘ and never analyzed, and rarely all markers are studied for a single cell. Additionally, as more and more cell populations can be detected, it becomes harder to identify which (combinations of) cell populations can be predictive for a clinical outcome.

In our lab, we developed the FlowSOM algorithm, which offers a comprehensive visualization of the data. FlowSOM makes use of a self-organizing map, making it computationally very scalable, and includes an additional meta-clustering step, allowing clusters in strongly varying sizes and shapes. Once the model is constructed, it can also be used to answer questions such as `What is the immunophenotypic difference between these two groups of patients?‘

FlowSOM is open source and available in R, and has recently been integrated as a plugin in FlowJo and Cytobank, making it easier accessible to wet lab scientists.

Workshop 2: Data analysis

(Un-)supervised learning of cell population structure from single-cell snapshot data

Mandfred Claassen

Institute of Molecular Systems Biology at ETH Zurich and Scailyte AG, Switzerland

Single-cell technologies have, in conjunction with unsupervised learning techniques, such as clustering, pseudotime ordering and bifurcation detection contributed extensively to the study of cell type heterogeneity.

Only recently, supervised learning techniques have been proposed to associate cell population heterogeneity with sample phenotypes such as clinical parameters.

We have established a representation learning approach to identify, possibly rare, disease associated cell populations in the context of cancer and immune biology. Specifically, we have developed CellCnn, a representation learning approach using convolutional neural networks to detect rare cell subsets associated with disease using high-dimensional single-cell measurements. Using CellCnn, we identified paracrine signaling-, AIDS onset- and rare CMV infection-associated cell subsets in peripheral blood, and extremely rare leukemic blast populations in minimal residual disease-like situations with frequencies as low as 0.01%. We extend CellCnn to associate imaging flow cytometry and histopathology images with disease phenotypes in T cell lymphoma and prostate cancer.

CellCnn is now enabling single cell studies in fundamental research and precision medicine, as for instance in the Swiss wide precision medicine initiative, that comprise single cell profiling of patient/sample cohorts each associated with specific disease statuses/phenotypes.

INFERENCE, a comprehensive mass cytometry analysis pipeline

Jan Verhoeff[1], Sophie Dusoswa[1], Sofie van Gassen[2], Juan J. Garcia-Vallejo[1]

[1]Amsterdam UMC - VUmc, Netherlands, The; [2]Data Mining and Modeling for Biomedicine, VIB Center for Inflammation Research, Ghent, Belgium

Cytomics is an emerging discipline that aims at the collective characterization and quantification of pools of cellular heterogeneity (the cytome) that translate into the structure, function, and dynamics of an organism. By taking a systems level approach, we can surface previously unknown regulatory circuits that could offer new diagnostics and therapeutic opportunities. We focus on the immune system cytome using mass cytometry-based multi-parametric single cell analysis together with bioinformatics in brain cancer. The immune system is a dynamic system of cells constantly undergoing stimulation, expansion, suppression, and cell death and cytomics provides us with in depth information on cell phenotypes, activation, and transitional states. Unfortunately, although bioinformatics tools allow for efficient cell clustering into recognizable immune cells subpopulations, they often do not provide for flexible statistical means for comparison. This pipeline aims at defining a building a flexible workflow to facilitate high dimensional cytomics analysis for immunologists.

We developed an analysis pipeline called INFERENCE to analyze mass cytometry data from

large cohorts of patients. Data from stained single cell suspensions of patients with differing disease state or diagnosis is acquired by mass cytometry (Helios, Fluidigm). After pre-processing, cells are clustered using FlowSOM (van Gassen et al, Cytometry A 2015) and an optimal number of subpopulations is determined using SPADEVizR (Gautreau et al, Bioinformatics 2017). The subpopulations are assigned phenotypes by median marker expression of populations in heatmaps and interpretation akin to classical gating strategies. Per patient the subpopulation frequencies are correlated against other subpopulations, revealing dynamic relationships between different cell types in network analysis software package Gephi (Bastian et al, Icwsm, 2009).

We first validated the INFERENCE pipeline using publicly available published datasets. We then applied INFERENCE to peripheral blood mononuclear cells obtained from a patient cohort of high and low grade gliomas with the aim of identifying disease-specific immune profiles. Our analysis yielded an unexpected and not previously recognized phenomenon in glioblastoma; namely high levels of a subset of B Cells. Further, correlation analysis revealed significant differences in the glioblastoma patients with regards to B and T cell subsets.

INFERENCE is a solid analysis pipeline that incorporates different algorithms to yield a flexible approach to cytomics amenable to use by immunologists without prior bioinformatics knowledge. While the correlation network analysis needs to be developed further, initial findings hold promise that it will add value to statistical comparisons.

Session 6

RNA viruses induce heterologous immunity against environmental allergens

Kathrin Balz[1], Hervé Luche[2], Renz Harald[1], Skevaki Chrysanthi[1]

[1]Institute of Laboratory Medicine and Pathobiochemistry, University Marburg;
[2]French Institute of Health and Medical Research, Paris, France

We have previously shown an influenza virus (IV) -mediated protective effect over subsequent development of experimental asthma in a murine model. This effect is mediated by CD44+ CD62L- T effector memory cells (Tem) that cross-react to allergen-and IV- derived T cell epitopes.

We aim on further characterizing such cross-reactive T cells at the single cell level using CyTOF technology. As a first step, CD45+ cells from naïve mice and ovalbumin (OVA) sensitized and challenged mice were isolated from lung and stained for a panel of surface markers ((CD11b, Ly6C, CD69, CD8a, CD27, CD25, CD3e, CD335, CD4, CD279, CD5, Klrg1, CD62L, CD44, NKG2ACE, CD45R, CD45.2) and cytokines (IL-5, IL-2, IL-22, IL-10, iNOS, TNFa, IFNy, IL-4, IL-6, TCRß, CD154, Perforin, GranzymeB, IL-17A) , providing a deep insight into the major immune cells subsets with a special focus on T memory cells. We could confirm an expected expression of cell surface markers and cytokine production patterns. This includes higher abundance of CD4+ and CD8+ T-effector memory cells in the OVA-treated group, but also higher numbers of regulatory T cells. Regarding cytokine expression, Th2 specific cytokines such as IL-4 and IL-5 were produced in significantly higher amounts compared to the control group.

Moreover, we compared two protocols for the handling of samples that need to be shipped. On the one hand, lung cells were stained directly after isolation, frozen, shipped on dry ice and measured at CIPHE in France. On the other hand, freshly isolated whole lungs were shipped on ice for 20 h, stained and measured at CIPHE in France. We could see significantly higher expressions of cytokines in the samples that have been stained directly and were frozen.

Therefore, we have established a protocol for validation of a panel of cell markers, including proper staining, freezing, shipping and data analysis workflow.

As a next step, we want to investigate the role of T-memory cells in Influenza-mediated asthma protection.

Furthermore, we are currently investigating the potential protective effect of other RNA viruses (respiratory syncytial virus and rhinovirus) in our experimental asthma model. Such samples will also be investigated in the future.

Next-gen immune profiling using imaging mass cytometry as a frame work for cancer immunotherapy

Marieke Ijsselsteijn[1], Vincent van Unen[2], Ruud van der Breggen[1], Arantza Farina-Sarasqueta[1], Frits Koning[2], Noel de Miranda[1]

[1]Department of Pathology, Leiden university medical centre, Leiden, The Netherlands; [2]Department of Immunohematology and blood transfusion, Leiden university medical centre, Leiden, The Netherlands

Immunotherapy has emerged as one of the most promising treatments for cancer. However, a substantial portion of patients are not responsive to current immunotherapies. Not only the mutational burden of tumours, but also the quality and quantity of tumour-infiltrating immune cells are an important prognostic indicator in several cancer types. Multiplex immunophenotyping technologies are essential to unravel the complexity of anti-tumour immune responses. Most techniques are held back by the lack of spatial context, limitations in the number of targets that can be visualised simultaneously, and/or cumbersome protocols. Imaging mass cytometry (IMC) overcomes these limitations by allowing for the visualisation of up to 40 markers using metal-conjugated antibodies combined with spatial information. Imaging mass cytometry is a versatile technique that can be utilised for the imaging of both fresh frozen and formalin-fixed paraffin-embedded (FFPE) tissue. The tissue of choice relies on factors such as the larger amount of validated antibodies for fresh frozen tissue against the better morphology that is obtained with FFPE. Furthermore, the use of FFPE requires that all antibodies function under the same antigen retrieval conditions.

We developed a 40 marker panel for the analysis of FFPE tissue which allows for extensive phenotyping by IMC. Next to a large quantity of immune cell markers, the panel consists of a number of structural and functional markers, to not only generate a comprehensive overview of the tumour microenvironment, but also to establish causal relations between immune and cancer cells. Furthermore, we created an optimised immunodetection workflow in which antibodies are split into two incubation steps, which reduces the concentration of total antibody per working-solution and employs the optimal incubation time and temperature for each antibody.

The 40 marker immune cell panel in combination with the for FFPE tissue optimised methodology will accelerate the identification of immune biomarkers with clinical relevance. Furthermore, this framework supports the discovery of previously unappreciated immune cell subsets, involved in anti-tumour immunity.

Fluidigm - Session: Imaging Mass Cytometry supported by Fluidigm
Investigating the Single Cell Pathology Landscape of Breast Cancer using Imaging Mass Cytometry

Jana Fischer[1,2]

[1]*Institute of Molecular Life Sciences, University of Zurich, Switzerland;*
[2]*Life Science Zurich Graduate School, ETH Zurich and University of Zurich, Switzerland*

Recent advances in spatially resolved single-cell analysis have revealed extensive tumor heterogeneity, yet most clinical decisions are still based on histologic stratification of tumors. Taking into account molecular subtypes (ER, PR, HER2) and cellular organization has improved breast cancer classification, enabling targeted therapy with a strong benefit for patients. However, intra-tumor heterogeneity and interactions between many more cell types may be responsible for therapeutic resistance and relapse. To extend the classic immunohistochemistry classification of breast cancer to the single-cell level, we segmented and analyzed high-dimensional Imaging Mass Cytometry (IMC) images. IMC of a 40-parameter antibody panel quantified tumor and stromal single-cell phenotypes, their interactions, and spatial heterogeneity in 300 breast cancers. Classification at the cellular level identified coexisting cancer cell phenotypes, and high and low risk subtypes within classic hormone receptor positive (HR+) and triple negative (TNBC) breast cancers. Here, single-cell pathology identifies cellular targets for future subtype-specific therapeutic intervention and provides a novel stratification of breast cancers with distinct clinical outcomes.

Single cell mass cytometry immunophenotyping linking genomics with proteomics in human systemic autoimmune diseases

Jozsef A. Balog[1], Ágnes Zvara[1], László Kovács[2], Attila Balog[2], László G. Puskás[1], Szebeni J. Gábor[1]

[1]*Biological Research Centre, Hungarian Academy of Sciences, Hungary;*
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Epidemiological data highlights the rising incidence of rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and systemic sclerosis (SSC) in the developed world over the last decades. Currently available treatments are palliative reducing the symptoms and supporting patient's wealth. Therefore, studies are much needed to deeply reveal cellular features responsible for pathologies or to identify early diagnostic and prognostic markers associated with RA, SLE and SSC, respectively.

Treatment-naive patients suffering either in RA, SLE or SSC who had not received non-steroidal anti-inflammatory drugs, disease modifying anti-rheumatic drugs and glucocorticoids until the time of blood sampling are enrolled aligned with age and gender matched healthy individuals. The whole transcriptome analysis of CD4+ T-cells is carried out in order to discover markers associated with RA, SLE or SSC. Cluster analysis dissects differential expression of genes in RA, SLE or SSC treatment naive patients compared to age and gender matched healthy controls. Potential diagnostic/prognostic proteins have been validated by the multiparametric and functional characterization of RA, SLE or SSC using single cell mass cytometry. Plasma proteome has been analysed by Luminex assay measuring 35 analytes per sample of RA, SLE or SSC patients and corresponding age and gender matched controls.

The pipeline of the whole transcriptome analysis, mass cytometry and plasma proteome profiling deeply reveals features of RA, SLE and SSC on the basis of highly multiplex phenotypical and functional characterization of drug-naive patients.

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Immune profiling of hepatocellular carcinoma indicates a clinical relevant role for exhausted T cells

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Hepatocellular carcinoma (HCC) is the major cause of liver cancer, a major global health problem with limited treatment options and poor prognosis. The immunobiology of hepatocellular carcnimoa is poorly understood and the immunogenicity of HCC is often considered to be relatively low. Exhausted T cells are increasingly recognized as a central feature of many cancers. They can recognize target cells but are functionally impaired due to inhibitory signals, such as by checkpoint receptors PD-1 and CTLA-4. We recently developed new tools facilitating the characterization of T cell exhaustion using transcriptomic, epigenomic or single-cell proteomic

signatures.

We set out to gain a better understanding of the immune response in general and T cell exhaustion in particular in HCC patients. Comprehensive profiling of the peripheral and intrahepatic immune compartment was performed using flow and mass cytometry. Transcriptome profiles from HCC resections were assessed for immune cell composition and the enrichment of exhausted T cell signatures and compared to patient survival.

Our preliminary data indicates that exhausted T cells can be identified in the peripheral blood and liver of HCC patients. The strong enrichment of exhausted T cells in the liver may represent a negative predictor for patient survival. Ongoing studies are focused on exploring different subsets of exhausted T cells that enrich in the tumor microenvironment and liver of patients with poor survival.

These data have implications for better understanding of T cell exhaustion in HCC required for successful immunotherapies.

High-dimensional cytometric analysis of colorectal cancer reveals novel and diverse mediators of anti-tumor immunity

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Checkpoint blockade has revived the potential of immunotherapy for cancer treatment. For optimal application and development of cancer immunotherapies, a comprehensive understanding of the anti-tumor immune response is required. We aimed at unravelling local and systemic immune profiles of colorectal cancer (CRC) by high-dimensional analyses to decipher the immune cell populations that participate in the process of tumorigenesis. The expression of 36 immune cell markers was simultaneously assessed at the single-cell level by mass cytometry in tumor tissues, tumor-associated lymph nodes, adjacent normal mucosa, and peripheral blood samples from CRC patients. In addition, functional and transcriptional profiles of tumor-infiltrating lymphocytes were investigated by flow cytometry and single-cell RNA-sequencing. We discovered that a previously unappreciated innate lymphocyte population (Lin-CD7+CD127-CD45RO+) is enriched in CRC tissues and displayed cytotoxic activity. This subset demonstrated a tissue-resident (CD69+, CD103+) phenotype, and was most abundant in the immunogenic mismatch repair deficient (MMRd) cancers. Furthermore, the presence of these Lin-CD7+CD127-CD45RO+ innate lymphoid cells strongly correlated with the presence of tumor-resident cytotoxic, helper and γδ T cells with a highly similar activated (HLA-DR+, CD38+, PD-1+) phenotype in CRCs. PD-1 intermediate and PD-1 high CD8+ T cell subsets represented distinct states of T cell activation and differentiation that further discriminated immunogenic (MMRd) from non-immunogenic (MMRp) CRCs. Remarkably, activated γδ T cells were specific for MMRd cancers, and their potential role in the response to PD-1 checkpoint blockade requires further clarification. The non-activated counterparts of the tumor-resident CD103+PD-1+ cytotoxic and γδ T cells were present in both tumor and healthy colorectal tissues. We did not detect any of the aforementioned tumor-resident immune cell populations in lymph node samples, with the exception of a tumor-positive lymph node. This indicates that the critical immune cell populations with anti-tumor activity reside in the colorectal mucosa, and that the role of lymph nodes in the anti-tumor immune response should be revisited in CRC. The findings presented here advance the

paradigm of anti-tumor immunity in CRC and provide a blueprint for the detailed characterization of the involved immune cell subsets. The coordinated action of innate and adaptive immune cell populations suggests a multi-targeted exploitation of their anti-tumor properties in a therapeutic setting.

A Statistical Approach of Estimating the Number of Clusters in SOM-clustering for CyTOF Data

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Masscytometry (CyTOF) offers the possibility to measure more than 30 parameters (still increasing) on single cell-level at the same time. To face the problem for cell-population detection in the resulting complex and high dimensional measured data, clustering approaches like hierarchical, kMeans, SOM or mixture-model clustering are widely used. Software-packages like CITRUS, SPADE, FlowSOM and immunoClust rely on either clustering-approach and offer feasible ways to analyze complex CyTOF data in order to retrieve cell-populations in cell-clusters and relate their features within a biological context.

Any clustering method requires initial settings and preselections to control the number of clusters. The number of clusters is either given in advance directly (kMeans, SOM), indirectly by a statistical parameter (immunoClust) or by a lower limit for the number of events for the clusters (hierarchical clustering). SOM-clustering showed a good performance for CyTOF data, but the choice for number of clusters is a guess. In practice several runs are performed and a “feel good” clustering result is selected. However, where are the limits for identification of new populations?

Estimating the number of clusters for a clustering approach leads to the statistical problem of selecting a model, which fits best to observed data. For this problem the Bayesian Information Criterion (BIC) was introduced by Schwarz (1978) and adjusted for mixture models by Biernackie et. al. (2002) with the Integrated Classification Likelihood (ICL).

We applied the ICL for estimating the number of clusters for the models retrieved by FlowSOM on a CyTOF dataset. The dataset consists 60 samples measured in 20 runs on CyTOF vs 1.5. Three CD45-barcoded samples were processed and acquired in parallel at a time. Data were normalized, manually de-barcoded on CD45 cells and asinh-transformed with a co-factor 5.

With FlowSOM a 20x20 grid based SOM was built and the varying models with K clusters were determined by consensus clustering using the resulting cluster labels to estimate means and co-variance matrices.

The resulting ICL-curve showed a maximum fairly close to the previously selected “feel good” selection for the number of clusters. This match of statistical and “feel-good” selection was even more convincing when the clustering was performed on preselected CD4 T-cell events only. The discontinuous dependency of ICL values from the number of clusters for the total dataset might be caused by not fully measured values. At the lower edge many values (ca. 50%) are below the detection limit and set to zero by CyTOF, which results in an inadequately estimation for the cluster co-variances.

In conclusion, applying ICL to CyTOF data enables to estimate an optimized number of clusters in a reproducible and statistically confirmed

manner. It also helps to estimate the limits of reliable separation of data into populations by clustering.

Effects of human cutaneous dissociation and preservation protocols onto epitope recovery and functional consequences on immune cells.

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Although CyTOF is currently scarcely used within the field of investigative dermatology, we aim to employ CyTOF's utilities in order to uncover the immunological heterogeneity and differentiation of resident versus non-resident immune cells in healthy and diseased human cutaneous tissues. However, the success of mass cytometry experiments depends on fully understanding the methods and how to control for variations when making comparisons between samples and different tissues. For example, the extraction of immune subsets from tissue is challenging, and the isolation technique used has often functional consequences on the cells obtained. Here, we tested experimental methods for human skin digestion and downstream CyTOF application that enable accurate data acquisition by optimizing signal detection and epitope recovery while minimizing background noise and low cell yields. We have tested on PBMCs and human acne inversa skin biopsies common fixation, freezing, mechanical and enzymatic digestion protocols while applying a newly established Mass Cytometry panel based on different T cell subsets with diverse tissue homing, cytotoxicity and effector potentials. We identified that digestion with Collagenase 1 or 4 with Benzonase for 6 hours at 37 oC not only maintain epitope stability for the selected markers in our panel but also do not alter cell viability while obtaining adequate cell yields. In contrast, Dispase II, which is often used in skin digestion protocols, digests even vital markers such as CD4, CD8, and CD56 and thus is not preferable for our applications. We conclude that many markers, such as chemokine receptors CXCR3, CCR6, CCR4, vital for phenotyping of immune subsets of our interest are highly sensitive to isolation and preservation methods. Therefore, optimization between cell yield and epitope recovery, based on the incubation time or enzyme selection, 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 and donors.

High-dimensional single-cell profiling of PBMCs in early MS using multiplexed mass cytometry

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Background: Multiple sclerosis (MS) is an inflammatory demyelinating and neurodegenerative disorder of the human brain. In rodents, experimental autoimmune encephalomyelitis (EAE) imitates the inflammatory demyelination of MS. Results obtained from this experimental model reveal high correlation of CNS infiltration of monocyte-derived macrophages to EAE severity. In human MS, the roles and impact of monocytes on MS pathogenesis and disease progression remain obscure. Here, we aim to determine the phenotypic alteration of monocytes and other circulating PBMCs in early MS patients and healthy individuals.

Methods: We performed comprehensive immune profiling of PBMCs in drug naïve early MS patients, in comparison to healthy donors (HD) using multiplexed mass cytometry. We determined the expression levels of 64 markers on PBMCs from healthy individuals and patients with early MS. Cell subsets of both, myeloid and lymphoid populations, are identified on two-dimensional (2D) t-SNE coordination. Immune phenotypes were comparatively determined between the groups (HD vs early MS). Finally, changes in cellular metabolism and function were assessed by flow cytometry and Seahorse analyzer, a live-cell metabolic assay platform.

Results: We detected an increased abundance of IL-6-expressing HLA-DR-CD33- lymphoid cells from early MS. These cells expressed high levels of CD47 and IKZF1. In the HLA-DR+CD33- cell population, a downregulation of HLA-DR expression was observed in B-cells from early MS, whereas in the same cell population, an increased CD19 expression could be detected. In contrast to the EAE model, we could not observe strong phenotypic alterations in HLA-DR+CD33+ monocytes/myeloid population from early MS patients. A slightly increased expression of CD95 and GATA6 was found in monocyte subsets from early MS patients. Finally, we detected a slight increase of mitochondrial respiration in early MS monocytes.

Conclusions: Herein, we provide the comprehensive phenotypic profiles of PBMC cell subsets in early MS patients compared to healthy individuals, which support the potential roles of peripheral adaptive and/or innate immunity in MS, suggesting target cell subsets for the preclinical and clinical studies on immune-dependent diagnosis and prognosis in MS.

Immune response to controlled malaria infection in malaria naïve protected Europeans using mass cytometry

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Malaria is a major health issue. Although drugs are available, it is still killing people worldwide, particularly in endemic regions. Therefore, much effort is put into developing a vaccine to prevent infection. The only well advanced malaria vaccine so far is RTS'S. However, it has partial protection (53%) against malaria in young children. There are now alternative vaccines being developed. A successful approach has been the development of attenuated parasites as vaccine. Attenuation can be either by irradiation of sporozoites or by chloroquine. In a recent study, protection was achieved by using the attenuation by chloroquine. To this end volunteers are put on chloroquine prophylaxis and are given either live P. falciparum sporozoites (vaccinated group) or saline (control group). This ensures that the parasites are killed but still induced a strong immunity. After 10 weeks of this vaccination period, the volunteers are exposed to malaria parasites. They are followed up to see if they develop parasitemia or not and, their PBMCs were collected. Here, we investigate the protection in 16 volunteers. The control group (5 out of 5 participants) all developed parasitemia, while 6/11 from the vaccinated group were not protected, 5 out of 11 showed strong protection. Samples (PBMCs) collected at c-1 (day 1 before CHMI) and d11 (11 days after the CHMI) were immunophenotyped using mass cytometry. Two panels of antibodies, one directed at phenotyping and the other to assess function by measuring cytokines, were applied.

Analyses are underway to identify immune subsets involved in protection against malaria and the cytokines that are produced.

Mass Cytometry Profiling of Signaling Pathways in SLE and During Therapeutic Intervention by JAK inhibitors

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Objective: Aberrant signaling in leukocytes contributes to the establishment and maintenance of chronic inflammation and autoimmunity. While the activity of signaling cascades and their abnormalities are known to be cell subset-specific, we lack a systems-level understanding of which immune cells signal aberrantly in different chronic inflammatory conditions and through which pathways. In this study, we compared peripheral blood leukocytes of Systemic Lupus Erythematosus (SLE) patients to healthy controls, both on the levels of cell subset abundance and protein phosphorylation. In addition, we monitored a small cohort of SLE patients treated over 16 weeks with the Janus kinase 1/2 inhibitor Baricitinib.

Method: A new sample collection workflow involving whole blood fixation and cryopreservation was established to arrest the ex vivo activation of leukocytes within 30 minutes after blood collection, providing access to cellular

activation states that are otherwise subject to time- or environment-dependent degradation. A 41-parameter mass cytometry panel was applied to these samples for simultaneous in depth-phenotyping of peripheral blood leukocyte subsets and interrogation of cell type-specific intracellular signaling on the levels of abundance and/or phosphorylation status of intracellular signal transducers.

Results: In comparison to healthy controls, SLE patients showed reduced frequencies of B cells, CD4+ T cells, basophils and dendritic cells in peripheral blood. Siglec1, a surrogate marker for type I interferon responses that correlates to lupus disease activity, was found to be up-regulated in CD14+ monocytes from SLE patients. Increased phosphorylation of STAT1 and 3 points towards a more activated phenotype of leukocytes in SLE. Aberrant pathway activation between diseased and healthy individuals could already be detected in ex vivo blood samples, without performing re-stimulation.

In our small patient cohort, Baricitinib was shown to ameliorate clinical symptoms in SLE. Reduction in skin manifestation and joint pain was paralleled by a decrease in Siglec1 expression on monocytes. Following similar kinetics, we observed a continuous decrease in STAT1 and 3 phosphorylation in CD4+ and CD8+ T cells, as well as CD14+ monocytes.

Summary and Outlook: In this proof-of-concept study we were able to show that mass cytometry is a valuable multiparametric screening tool for monitoring immunophenotypic and signaling-related, molecular changes in chronic inflammatory diseases. Using phospho-specific antibodies, we were able to detect cell signaling aberrances ex vivo in cryopreserved whole blood samples. The cell-based results will now be combined with serum cytokine analyses to obtain a more comprehensive picture of important factors in the peripheral blood of patients suffering from chronic inflammatory disease. It can be expected that these data will provide valuable new insights into disease pathogenesis and single cell-based signaling processes targeted by a new class of small molecule therapeutics in the field of chronic inflammatory diseases.

Methods for whole blood immunomonitoring of clinical samples for flow and mass cytometry studies.

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Background: PRECISESADS is a multicenter project that aims at the molecular re-classification of the systemic autoimmune diseases combining molecular and cellular -omics. To obtain cytomic data patient's peripheral blood samples are collected across different research centers for flow and mass cytometry analysis. Therefore to avoid center bias and technical variations we considered the preservation of collected samples until the time of staining and analysis. However, current available methods for whole blood preservation in cytometry studies have not been systematically compared. We would like to fill this gap by providing useful information for the appropriate method selection for both conventional and mass cytometry.

Methods: Herein we compare two buffers for whole blood fixation and cryopreservation: Smart Tube Proteomic Stabilizer (PROT1) and BD phosphoflow lyse/fix buffer (BD). Eight surface markers were verified using flow cytometry, and 15 surface and 8 intracellular markers were verified for mass cytometry upon R848 stimulation in fresh blood, and compared with the results obtained after fixation and freezing. The verified storage time was 1 week, 1 month and 6 months for both flow and mass cytometry.

Results: Whole blood can be preserved and stored until acquisition, however care needs to be taken when designing the antibody panel. In flow cytometry BD buffer allows monocyte/granulocyte discrimination based on FSC/SSC, while with PROT1 a granulocyte or monocytes marker is needed. Granulocytes cannot be phenotyped with confidence by flow cytometry due to their unspecific antibody binding to fluorochrome-conjugated antibodies but not to metal-conjugated antibodies in both fixation methods. In both techniques similar percentages of major leucocytes and their subpopulations were observed when comparing the two fixation methods to the unfixed condition. A cytokine response study can be done using both buffers, as we did not observe significant differences between two products by using mass cytometry.

Conclusions: Preservation will allow deep phenotyping provided that the choice of buffer is made according to the importance of particular markers, clones of antibodies and populations. Due to higher background of fluorochrome-conjugated antibody phenotyping studies are more challenging than in the case of metal conjugated antibodies. We consider blood preservation as a good method for minimizing center bias and technical variation in both flow and mass cytometry studies.

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Multiplexed single-cell profiling of signalling in patient-derived colorectal cancer organoids

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The intestinal epithelium is a highly organised tissue consisting of cell type hierarchies. In colorectal cancer progression, this cellular organisation is eventually disrupted. However, it remains unclear if and how tumours retain aspects of the intestinal cell hierarchies, how oncogenic mutations shift these hierarchies and how phenotypic heterogeneity within tumours influences drug response. Recent convergences of high-throughput technologies and single-cell assays now allow quantification of cell-by-cell variabilities in a system-wide manner. Furthermore, patient-derived 3D cultures make it possible to model and perturb tumour tissue in vitro. Here, we leverage mass cytometry and scRNA-seq, to profile cell signalling dynamics and the resulting gene expression changes on a cell-by-cell basis in a panel of patient-derived colorectal cancer organoids (PDOs). In an integrative approach, we identify both cell type-specific signalling and gene expression dynamics, and computationally reconstruct spatio-temporal hierarchies of the identified cell types. To achieve parallel profiling of different PDOs, we employ sample multiplexing and SNP-based deconvolution of samples which allows us to identify patient-specific differences in cellular organisation.

Comparative study on the impact of different cryo-storage methods on staining and cellular composition using mass cytometry

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Freezing blood samples is a routine procedure in basic and clinical research for storage or shipment. Although classical procedures allow cryopreservation of peripheral blood mononuclear cells, it is now possible to freeze whole blood using commercialized reagents, therefore enabling simple conservation of all leukocytes. Several reports investigated the effect of different freezing and thawing methods on peripheral blood composition, viability or staining quality. However, a comprehensive study using cellular profiling is still missing.

In this study, the impact of three cryo-storage methods on cell composition and staining quality will be compared using Mass Cytometry and a panel of 37 antibodies. These methods are: 1) 90%FCS, 10%DMSO and 2) 45%RPMI, 45%FCS, 10%DMSO for PBMCs freezing and 3) the SmartTube System for whole blood or PBMCs. Furthermore, the datasets will be analyzed using different bioinformatic pipelines. The differences in results obtained with manual gating, FlowSom or ACISE (Automated Comprehensive Immunophenotyping and Subset Enumeration) will then be evaluated.

The preliminary results comparing frozen/thawed cells with the SmartTube system and fresh PBMCs were analyzed with different bioinformatics tools. Major differences in staining were found, particularly regarding chemokine receptors and monocyte subpopulations. We hypothesize that this is an effect of the cryo-storage and/or of the fixation induced by the SmartTube Proteomic Stabilizer buffer.

In the future, we aim at comparing the three methods of cryo-storage on PBMCs and the SmartTube system only on whole blood with fresh whole blood or PBMCs from 5 healthy donors. However, another experiment to test all the methods of cryo-storage and compare them to fresh blood will be conducted at the beginning of next year.

Furthermore, the panel of antibody we are currently using is staining only surface markers. If this panel does not allow for proper subpopulation separation and identification after freezing, we will investigate alternative intracellular markers to enable a more complete analysis.

Maximizing human immune monitoring studies with mass cytometry

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Immune monitoring is an essential method for quantifying changes in immune cell populations in chronic inflammation, infectious disease, autoimmune disease and cancer studies. The extreme heterogeneity of immune cells demands a high-parameter approach to more fully and efficiently quantify the immune response in health and disease. Mass cytometry is an ideal solution, enabling the simultaneous detection of over 40 phenotypic and functional markers in a single tube of sample. We report development of a 29-marker panel for mass cytometry based on the Human ImmunoPhenotyping Consortium (HIPC) consensus panel [Maecker et al. Nature Reviews Immunology (2012)], expanded to allow identification of additional leukocyte subsets, particularly T cells. Automated data analysis with Verity Software House GemStone™ software has been developed specifically for data collected with the panel. Extensive panel testing for repeatability, reproducibility and agreement of full versus partial panel population identification was performed. Repeatability was tested with a single PBMC sample stained by a single technician in two technical replicates and acquired in triplicate on two Helios™ mass cytometers. SDs for percent of parent were 1% or less for 16 identified populations. Reproducibility was tested by determining the variability in measurements of five PBMC lots stained by five technicians and collected on two Helios instruments. CVs on mean percent of 13 populations were under 15% for all but three of 130 measurements. Lastly, R2 values for agreement of percent parent populations using the full 29 marker panel compared to a 10 marker panel for T cell populations were 0.94 or higher. We conclude that this panel kit can provide consistent immune population identification and enumeration for any given lot of PBMC.

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