<table>
<thead>
<tr>
<th>Time</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:15 - 8:45</td>
<td>Registration</td>
</tr>
<tr>
<td>8:45 - 9:00</td>
<td>Welcoming session</td>
</tr>
<tr>
<td>9:00 - 10:00</td>
<td>Opening keynote session</td>
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<tr>
<td></td>
<td>Senior PI: Prof. Dr. Federica Sallusto (ETH)</td>
</tr>
<tr>
<td>10:00 - 10:30</td>
<td>Talk 1: Dr. Milena Sokolowska (SIAF)</td>
</tr>
<tr>
<td>10:30 - 11:00</td>
<td>Coffee break</td>
</tr>
<tr>
<td>11:00 - 11:30</td>
<td>Talk 2: Dr. Laura Cadarri (Roche)</td>
</tr>
<tr>
<td>11:30 - 12:00</td>
<td>Talk 3: Dr. Giuseppe Locatelli (UniBern)</td>
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<tr>
<td>12:00 - 13:00</td>
<td>Lunch break</td>
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<tr>
<td>12:30 - 13:00</td>
<td>Poster session 1 (even poster numbers)</td>
</tr>
<tr>
<td>13:00 - 14:00</td>
<td>Prof. Dr. Christian Münz (EJI)</td>
</tr>
<tr>
<td>14:00 - 15:00</td>
<td>Workshops in parallel</td>
</tr>
<tr>
<td>15:00 - 16:00</td>
<td>Coffee break</td>
</tr>
<tr>
<td>15:00 - 15:30</td>
<td>Poster session 2 (odd poster numbers)</td>
</tr>
<tr>
<td>16:00 - 16:30</td>
<td>Talk 4: Prof. Dr. Emma Slack (ETH)</td>
</tr>
<tr>
<td>16:30 - 17:30</td>
<td>Closing keynote session</td>
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<tr>
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<td>Young PI: Dr. Daniela Latorre (ETH)</td>
</tr>
<tr>
<td>17:30 - 18:00</td>
<td>Poster prize announcement</td>
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<tr>
<td>18:00 - 20:00</td>
<td>Apéreo</td>
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</table>

**Location:** ETH Zentrum HG F30 – Audi Max

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Keynote speakers

Senior PI Keynote talk

Federica Sallusto
ONE FOR ALL: CROSS-REACTIVE T CELLS AND ANTIBODIES TO CORONAVIRUS
9.00-10.00

Young PI Keynote talk

Daniela Latorre
AUTOACTIVE T CELLS IN NEUROLOGICAL DISORDERS
16.30-17.30
Rhinoviruses (RV) and allergens, such as house dust mite (HDM) are major agents responsible for asthma exacerbations. The influence of pre-existing airway inflammation on the infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is largely unknown. Here, we described that RV infection in patients with asthma led to an excessive engagement of RIG-I with ASC and caspase-1 into inflammasome formation which diminished RIG-I accessibility for TBK1/IKKe/IFN-I/III responses, leading to their early functional impairment, delayed resolution, prolonged viral clearance and unresolved inflammation in vitro and in vivo. Pre-exposure to HDM augmented this phenomenon by priming for pro-IL-1β and auxiliary inhibition of early IFN-I/III responses. Prior infection with RV restricted SARS-CoV-2 replication, but co-infection augmented RIG-I inflammasome activation and epithelial inflammation. Timely inhibition of the epithelial RIG-I inflammasome and reduction of IL-1β signaling may lead to more efficient viral clearance and lower the burden of RV and SARS-CoV-2 infection.


In Roche we are developing several next generation PD1-based antibodies with different properties and mechanism of action. I will present a few approaches we used to simultaneously block PD-1 together with additional immune checkpoints. These molecules maintain tumor-specific T cell functionality and prevent tumor-escape. I will also present our seminal work on PD-1 targeting as a means to selectively deliver cytokines to tumor-specific T cells to drive their expansion, differentiation and anti-tumor activity.
Scientific Talks

Talk 3

Giuseppe Locatelli

IN VIVO DYNAMICS OF NEUROINFLAMMATION

11.30-12.00

Pathologies such as multiple sclerosis and its animal model experimental autoimmune encephalomyelitis are characterized by chronic inflammation and concomitant neurodegeneration. Intravital imaging in the murine central nervous system is a powerful tool allowing to shed light on this complexity. In this talk, I will illustrate the functional dynamics of resident and invading immune cells, as well as the underlying damaging mechanisms affecting oligodendrocytes and myelin.

Talk 4

Emma Slack

GUT FEELINGS ABOUT MICROBIOTA ENGINEERING

16.00-16.30

When all is well in your gut microbiota, you will barely notice their existence, although these microbes carry out a wide range of functions critical to your health. When opportunistic species overgrow, or critical functions get lost, then these same microbes can make your life hell. In this talk we will explore the mechanisms that help to maintain healthy microbiome function and how immune mechanisms in the intestine can be manipulated to permit rational microbiota engineering.
Meet the Editor

Christian Münz

AN EDITOR’S GUIDE TO SCIENTIFIC PUBLISHING
13.00-14.00

Christian Münz (EJI Executive Committee) will give writing tips and discuss what editors are looking for in a manuscript. Christian will also guide you through the peer review process with some examples of editorial decisions, and discuss any questions you may have in the dedicated Q&A section of this session.
The ability to acquire research funding is an important building block of an academic career. A perfect proposal is not only scientifically excellent. There are a lot of other factors that influence the decision of an evaluation committee. In this workshop, we will have a look at frequent rejection reasons and discuss how these can be mitigated. Reflection on criticism can help you improve your own future proposals.

This workshop provides you with 20 real-life, practical tips to instantly improve your public speaking skills. Developed by the Swiss/American Entertainer and Cartoonist David Levine, this one-hour workshop will help you to rock your next presentation. David Levine, "Crazy David" is a freelance cartoonist and entertainer. Born in the USA, he has lived in Bern since the early 1990's, drawing caricatures for diverse media and private as well as corporate clients. David developed his public speaking skills through years of teaching Art and English to audiences between the ages of 4 and 99. At the end of this workshop, each participant will receive a free, signed comic-book containing all the public speaking tips.

My journey from Academia to Biotech via "Big Pharma": connecting the dots. During the workshop we will discuss about choices, opportunities, doubts and myths that a young scientist has to face at early stage of his/her career.
Posters
TCR-QR: Identifying tumour-reactive TCRs for personalized T cell therapies

Florian Bieberich¹, Rodrigo Vazquez-Lombardi¹,², Marta Trueb³, Marcel Trefny³, Alfred Zippelius³, Sai T. Reddy¹

¹Department of Biosystems Science and Engineering, ETH Zurich, Basel, Switzerland
²EngImmune Therapeutics, Basel, Switzerland
³Department of Biomedicine, University Basel, Basel, Switzerland

Personalized immunotherapies such as adoptive cell transfer (ACT), from expanded tumor-infiltrating lymphocytes (TILs) have shown promising results across different tumor entities. Nonetheless, while it mediates complete regression in a fraction of some patients, many patients lack response to TIL therapy.

One potential reason is that expanded TIL products include both tumor-reactive and bystander T cells, which thus may be leading to a lack of therapeutic efficacy and potency. Increasing the number of tumor-reactive TILs would substantially advance the efficacy of such personalized therapies. However, it is highly challenging to identify tumor-reactive TILs - and even more so in the limited timeframe needed for most personalized immunotherapies.

Here, we use multi-dimensional data from single-cell transcriptome, epigenome, immune repertoire and functional reactivity assays to identify and eventually predict tumor-reactive TCRs.

By introducing selected TCRs from TILs into our previously developed TCR testing platform (TnT, Vazquez et al. 2021 under revision at Cell), we identified enrichment of a subset of TCRs that showed expression of phenotypic markers that partly overlap with established markers of exhaustion and tumor reactivity such as CD39, CXCL13 and CD137. Combination with single cell chromatin accessibility sequencing (scATACseq) allowed us to project motif accessibility for TILs of interest and showed elevated motif activity of TCF7 and NFKB1. Future experiments will increase numbers of patient as well as tested TCRs per patient.

In conclusion, TCR-QR consists of an integrated pipeline that exploits state-of-the-art methods in single-cell genomics, computational analysis, genome engineering and functional screening. This allows for new marker identification and patient sample integration on the multiOMIC level.
Intercrypt Sentinel Macrophages Induce a Tunable Antibacterial NFκB Response

Annika Hausmann1, Boas Felmy1, Leo Kunz2, Sanne Kroon1, Dorothée L. Berthold1, Giverny Ganz1, Ioana Sandu1, Toshihiro Nakamura1, Nathan Zangger1, Yang Zhang2, Tamas Dolowschiak1, Stefan A. Fattinger1,3, Markus Furter1, Anna A. Mueller Hauser1, Manja Barthel Scherrer4, Katerina Vlantis4, Laurens Wachsmuth6, Jan Kisielow5, Luigi Tortola5, Danjiela Heide6, Mathias Heikenwaelder6, Annette Oxenius1, Manfred Kopf5, Timm Schroeder2, Manolis Pasparakis4, Mikael E. Sellin1,3, Wolf-Dietrich Hardt1.

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3Department of Medical Biochemistry and Microbiology, Uppsala Universitet, Uppsala, Sweden
4CECAD, University of Cologne, Cologne, Germany
5Institute of Molecular Health Sciences, D-BIOL, ETH Zurich, Zurich, Switzerland
6Division of Chronic Inflammation and Cancer, German Cancer Research Center, Heidelberg, Germany

Intestinal epithelial cell (IEC) NFκB signaling regulates the delicate balance between mucosal homeostasis and inflammation. It is not fully understood which signals tune this balance, and how bacterial exposure elicits the process. Pure LPS induces epithelial NFκB activation in vivo. We found that in mice, IECs do not respond directly to LPS. Instead, tissue resident intercrypt lamina propria sentinel macrophages sense LPS via TLR4 and rapidly secrete TNF to elicit epithelial NFκB signaling in their immediate neighborhood. This response is relevant also during oral enteropathogen infection. The macrophage-TNF-IEC axis avoids responses to luminal microbiota LPS, but enables localized or tissue-wide epithelial NFκB responses in proportion to the microbial threat. Thereby, intercrypt macrophages serve as important first-responding sentinels for Gram-negative microbes breaching the epithelial barrier. The tunability of this crypt response allows induction of defense mechanisms at an appropriate scale according to localization and intensity of microbial triggers.
ACE2 engagement exposes the fusion peptide to pan-coronavirus neutralizing antibodies

Jun Siong Low1,2*, Josipa Jerak1,2†, M. Alejandra Tortorici3†, Matthew McCallum3†, Dora Pinto4‡, Antonino Cassotta3, Mathilde Foglierini3, Federico Mele3†, Rana Abdelnabi5, Birgit Weynand6, Julia Noack7, Martin Montiel-Ruiz7, Siro Bianchi4, Fabio Benigni4, Nicole Sprugasci4, Anshu Joshi3, John E. Bowen3, Alexandra C. Walls3,8, David Jarrossay1, Diego Morone1, Philipp Paparoditis1, Christian Garzoni9, Paolo Ferrari10,11,12, Alessandro Ceschi10,13,14,15, Johan Neyts5,16, Lisa A. Purcell7, Gyorgy Snell7, Davide Corti4, Antonio Lanzavecchia4,17§, David Veesler3,8*§ and Federica Sallusto1,2*§

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* Corresponding authors
†,‡,§ Equal contribution

Coronaviruses use diverse Spike (S) glycoproteins to attach to host receptors and fuse with target cells. Using a broad screening approach, we isolated from SARS-CoV-2 immune donors seven monoclonal antibodies (mAbs) that bind to all human alpha and beta coronavirus S proteins. These mAbs recognize the fusion peptide and acquire high affinity and breadth through somatic mutations. Despite targeting a conserved motif, only some mAbs show broad neutralizing activity in vitro against alpha and beta coronaviruses, including Omicron BA.1 variant and bat WIV-1, and reduce viral titers and pathology in vivo. Structural and functional analyses show that the fusion peptide-specific mAbs bind with different modalities to a cryptic epitope which is concealed by prefusion-stabilizing ‘2P’ mutations and becomes exposed upon binding of ACE2 or ACE2-mimicking mAbs. This study identifies a new class of pan-coronavirus neutralizing mAbs and reveals a receptor-induced conformational change in the S protein that exposes the fusion peptide region.
Interplay between gut microbiota, specific IgA and tumor-associated neutrophils in human colorectal cancer

Elisa Sorrenti¹, Camilla Basso¹, Julija Djordjevic¹, Laura Terzaghi¹, Jacopo Galafassi¹, Dimitri Christoforidis¹, Pietro E. Majno-Hurst¹, and Giandomenica Iezzi¹.

¹Laboratories for Translational Research, Department of Surgery of Ente Ospedaliero Cantonale and Università della Svizzera Italiana

Introduction: Colorectal cancer (CRC) infiltration by tumor-associated neutrophils (TANs) is associated with prolonged patients’ survival, but underlying mechanisms remain unclear. Recent studies have reported anti-tumoral activity of TANs upon triggering of CD89 by immunocomplexes (ICs) including IgA, whose production largely depends on gut microbiota. In CRC, microbiota translocate across the neoplastic epithelium and interact with tumor and infiltrating immune cells. In this project we want to investigate whether ICs formed by translocated microbiota and bacteria-specific IgA may trigger TAN antitumor functions.

Method: CD89 expression was evaluated by flow cytometry on, cell suspensions, obtained from freshly isolated samples of CRC and tumor-free colonic tissues upon enzymatic digestion, and on autologous peripheral blood mononuclear cells (PBMC) upon staining with specific antibodies. Total and bacteria-specific IgA levels in plasma of CRC patients were evaluated by ELISA.

Results: In preliminary results, CRC tissues displayed higher densities of CD89+ neutrophils as compared to the corresponding tumor-free tissues. IgA specific for most abundant bacteria in human CRC, i.e. Fusobacterium nucleatum and Bacteroides fragilis, were detectable in patients’ plasma.

Conclusions: Commensal-bacteria specific IgA are found in plasma of CRC patients and may bind to CD89 expressed on TAN. We are currently evaluating effects elicited in neutrophils by IgA-microbiota IC in vitro. Furthermore, we are establishing an orthotopic CRC mouse model in hCD89 transgenic mice to assess the antitumor potential of IC-activated TAN in vivo.
The effect of a Ketogenic Diet on the host Microbiota, the Immune System and the CNS

Andrina Rutsch¹,², Johan Björn Kantsjö³,², Francesca Ronchi F¹,².

¹University of Bern, Department of Visceral Surgery and Medicine, Inselspital, Bern University Hospital, University of Bern, 
²Charité Universitätsmedizin Berlin, Campus Benjamin Franklin, Institut für Mikrobiologie und Infektionsimmunologie

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The microbiota can shape the CNS through bacterial products and it is highly influenced by different environmental factors, like the diet. Ketogenic diet (KD) ameliorates metabolic and neurological conditions. However, the mechanisms behind it are not well known.

My aim is to understand the cellular and molecular effects of KD on the composition and function of the gut microbiota and subsequently the CNS under healthy and neurological disease conditions. To study the microbiota, I performed 16S rRNA sequencing, biomass measurements and metatranscriptomics on the intestinal microbes of mice fed KD or a control diet (CD) under different hygiene conditions (specific-pathogen free (SPF) and a defined microbiota (sDMDMm)). KD induced increase in Ruminococcaceae, Erysipelotrichaceae and Clostridiales, and decrease in Bacteroides and Prevotellaceae families, with important metabolic changes.

To investigate the effect of KD on the host immune and nervous system in a microbiota-dependent and -independent manner, I analyzed intestinal and brain immune cells by flowcytometry and the brain nervous system by spatial transcriptomics. My preliminary results revealed a decrease in γδT cells in the brain of SPF mice fed KD compared to control groups (including germ-free mice). My experiments provide important findings in the effects of KD on the microbiota, immune cell and brain function.
Dynamics of T Cell Repertoire Renewal Following Autologous Hematopoietic Stem Cell Transplantation in Multiple Sclerosis

Josefine Ruder¹, María José Docampo¹, Jordan Rex¹, Simon Obahor¹, Antonia M.S. Müller², Urs Schanz², Ilijas Jelcic¹, Roland Martin¹*.

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Autologous hematopoietic stem cell transplantation (aHSCT) is a highly effective treatment of multiple sclerosis (MS). It involves mobilizing hematopoietic stem and progenitor cells (HSPCs), destroying the immune system by high-dose chemotherapy, and subsequently reinfusing autologous HSPCs for immune reconstitution. Prior studies showed that this procedure depletes autoreactive cells and leads to subsequent renewal of adaptive immune cells. However, surviving T cells have been described, but their dynamics and possible proinflammatory potential early (≤ 6 months) after aHSCT have not been studied. Here, we aim to understand the dynamics and extent of new and surviving T cells after aHSCT in MS. In 27 patients, we applied multidimensional flow cytometry, T cell receptor (TCR) sequencing, telomere length profiling, and HLA-genotyping to address this question. Early post-aHSCT, naïve T cells are barely detectable, while effector memory (EM) T cells quickly reconstitute to pre-aHSCT levels. EM CD4+ T cells early post-aHSCT show shorter telomeres and higher expression of senescence- (CD57+, CD27-, CD28-) and exhaustion markers (PD1+, CD39+, TIM3+) compared to pre aHSCT. We find a median TCR repertoire overlap of 26% between the early post-aHSCT EM CD4+ T cells and pre-aHSCT, indicating broad persistence of EM CD4+ T cells early after transplantation. This EM CD4+ TCR repertoire overlap declines to a median of 15% at 12 months post-aHSCT, while the naïve TCR repertoire entirely renews, showing a median overlap of only 0.1%. Our data support the complete renewal of the T cell repertoire by nascent T cells late after aHSCT, while substantial survival of pre-aHSCT EM CD4+ T cells is evident in the early phase.
Poster 7

Geometry, affinity, and forces: understanding the constraints at play in robust B-cell responses against bacterial glycans

Suwannee Ganguillet, Yagmur Turgay, Stefanie Oswald, Milad Radiom, Emma Wetter-Slack.

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High-affinity IgA confers protection against Salmonella by enchaining growth and can be achieved by target-specific oral vaccination. Bacterial surface glycans are ubiquitously expressed and dominant protective antibody responses in E. coli and Salmonella infections, two common bacterial enteropathogens. However, while systemic immune responses against proteins and haptens are well studied, little is known about efficient mucosal priming and glycan-antigen processing. Here, we develop two novel B-cell receptor knock-in mouse lines showing 1000-fold differences binding affinities to six Salmonella Typhimurium O-antigens variants and exhibiting different biochemical and biophysical properties. Using whole bacteria immobilisation, lipopolysaccharide-integrated artificial planar lipid bilayers, and in-vivo oral immunization, we examine the effects of glycan length, flexibility and structure on antigen sampling, B-cell priming and affinity maturation. Combined with live microscopy and adoptive transfer, we can accurately track the localisation of activated B-cells following different modes of vaccinations and investigate the recruitment and limitations of T-cell help. This will generate a novel, comprehensive and fundamental understanding of mucosal responses to glycan antigens that can be applied to improve rational vaccine design, offering a robust alternative to antibiotics.

The C5a-C5aR1 complement axis is essential for neutrophil recruitment to draining lymph nodes via high endothelial venules in cutaneous leishmaniasis

Borja Prat-Luri¹, Christopher Neal¹, Katiuska Passelli¹, Emma Ganga¹, Jonas Amore², Luan Firmino-Cruz¹ †, Tatiana V. Petrova³,⁴, Andreas J. Müller² and Fabienne Tacchini-Cottier¹

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Neutrophils are innate immune cells known for their ability to fight pathogens. Their trafficking throughout the body is key to perform their functions. However, the mechanisms of neutrophil trafficking to lymph nodes are not fully clear. Using a murine model of dermal infection with Leishmania parasites, we observe a transient neutrophil influx in draining lymph nodes despite sustained recruitment to the infection site. Cell tracking experiments, together with intravital two-photon microscopy, indicate that neutrophil recruitment to draining lymph nodes occurs minimally through lymphatics from the infected dermis but mostly through blood vessels via high endothelial venules. Mechanistically, neutrophils are guided by the C5a-C5aR1 axis to extravasate into the draining lymph node parenchyma. We also report that C5, the C5a precursor, is locally produced in the draining lymph node by lymphatic endothelial cells. Our data establishes and details organ-specific mechanisms of neutrophil trafficking.
Influence of gut microbiota and diet on the onset and course of EAE in RR mice, colonised with microbes from multiple sclerosis patients

Johan Björn Kantsjö1,2, Andrina Rutsch1,2, Friedemann Paul3 & Francesca Ronchi1,2

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2 Charité Universitätsmedizin Berlin, Institute of Microbiology, Infectious Diseases and Immunology, Germany
3 Charité Universitätsmedizin Berlin, NeuroCure Clinical Research Center (NCRC), Germany

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Multiple sclerosis (MS) is an autoimmune devastating disease with increasing occurrence in “westernised” countries. This has been associated with changing life-style factors i.e. diet, hygiene and microbiota. The microbiota differs significantly between healthy people and MS patients, as well as between different phases of the disease (1). In the MS murine model, experimental autoimmune encephalomyelitis (EAE), the absence of the microbiota is associated with no disease development in mice with a high proportion of myelin-specific T cells (2).

The aim of my project is to understand these interrelations and how the microbiota, upon diet change, could affect the development of the disease in animal models. Faecal samples from MS patients at diagnosis were transplanted into relapsing-remitting (RR) TCR1640 mice, bearing a MOG-specific T cell receptor. These mice spontaneously develop EAE, which will enable to study influences of diet and microbiota on the onset and course of MS in vivo. We will compare the influence of a western-like diet (high cholesterol & sugar) to a healthier Mediterranean diet, on the host microbiota and immune responses during different phases of the disease. With bacterial isolation and identification we hope to determine specific species associated with progression or alleviation of MS under different dietary regimens and their effect on the host immunity.

The pulmonary endothelium arms Natural Killer cells for anti-metastatic responses

Marijne Vermeer¹, André Fonseca da Silva², Colin Sparano³, Tobias Wertheimer², Caroline Mussak¹, Dario Solís-Sayago¹, Gioana Litscher¹, Maud Mayoux¹, Burkhard Becher², Lothar C. Dieterich³ and Sônia Tugues¹.

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The lung is a common site for metastasis for many patients suffering from different tumor types. The success of metastatic growth is determined by the continuous interactions tumor cells establish with a wide range of immune cells. Natural Killer (NK) cells play a key role in inhibiting lung metastasis. It is however not clear when, where and how NK cells control metastatic tumor cells extravasating to the lung tissue. In this study, we found that the elimination of metastasizing tumor cells by NK cells in the lung is a very rapid process that takes place intravascularly. As such, NK cells interact with the pulmonary endothelium through the integrins Lymphocyte Function-associated Antigen 1 (LFA-1) and Very Late Antigen (VLA-4), which bind to endothelial ICAM-1 and VCAM-1, respectively. Targeted disruption of the integrin-mediated NK cell interaction with the pulmonary endothelium leads to increased apoptosis and a decrease of mature lung NK cells. Our findings suggest that the crosstalk between NK cells and endothelium may enable NK cells to survive and mature, so they can perform the necessary functions for tumor elimination. Modulating this interaction may be key for the development of lung-specific antimetastatic therapies.
**Poster 11**

*Exocrine gland-resident memory CD8⁺ T cells use mechanosensing for tissue surveillance*

Nora Ruef¹, José Martinez Magdaleno¹, Xenia Ficht², Vladimir Purvanov³, Matthieu Palayret¹, Stefanie Wissmann¹, Petra Pfenninger¹, Bettina Stolp⁴, Flavian Thelen⁵, Juliana Barreto de Albuquerque⁶, Philipp Germann⁷, James Sharpe⁷,⁸,⁹, Jun Abe¹, Daniel F. Legler³,⁹,¹¹, Jens V. Stein¹,⁹,¹¹.

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¹⁰Faculty of Biology, University of Konstanz, 78464 Konstanz, Germany
¹¹Theodor Kocher Institute, University of Bern, 3011 Bern, Switzerland

Tissue-resident CD8⁺ T cells (T RM) constitutively scan peptide-major histocompatibility complexes (pMHC) in their organ of residence to intercept microbial spread. While chemokines and integrin ligands produced at epithelial barriers are critical mediators of this process, their elevated constitutive expression in uninfected non-barrier organs might lead to excessive influx of immune cells. We recently found that exocrine gland T RM are programmed for autonomous tissue scanning in the absence of any chemoattractant or adhesion receptor engagement. The signals eliciting this non-canonical motility mode and its relevance for organ surveillance have remained unknown. Here, we report that exocrine gland T RM autonomously generated retrograde F-actin flow for locomotion, accompanied by high Myosin IIA-dependent cortical contractility and leading edge bleb formation. The distinctive mode of exocrine gland T RM locomotion was triggered by sensing physical parameters of its microenvironment, and closely correlated with nuclear deformation, which acts as a mechanosensor via an arachidonic acid and Ca²⁺-signaling pathway. In contrast, naïve CD8⁺ T cells or small intestine T RM did not show mechanosensing capacity. Inhibition of the nuclear mechanosensing disrupted exocrine gland T RM scanning and impaired their ability to intercept target cells. In sum, mechanosensing of physical confinement suffices to elicit homeostatic T cell surveillance of exocrine glands, and acts to complement chemosensing-mediated migration in non-inflamed organs.
Intrahepatic priming generates long-lived but dysfunctional TRM-like CD8+ T cells

Caroline Claire Krüger1,2, Chiara Laura1,2,3, Xenia Ficht1,2, Matteo Iannacone1,2.

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CD8+ T cell priming can occur in the liver during neonatal HBV infection, resulting in activation, proliferation and generation of dysfunctional CD8+ T cells. Preliminary bulk RNA sequencing has shown that this dysfunction is different from classical T cell exhaustion. However, it is unknown whether these dysfunctional cells are homogeneous or whether subpopulations exist. To increase the granularity, we employed single-cell RNA sequencing to determine the heterogeneity, relationships of the subsets and to identify new targets for immunotherapeutic intervention.

Here we show that intrahepatically primed CD8+ T cells persist for at least 6 months. A small subpopulation with stem-like phenotype was uncovered at day 28, which is characterized by the expression of stem-like marker genes such as Tcf7 and Slamf6 and genes related to T cell exhaustion like Tox or inhibitory receptors. Nevertheless, most cells at day 28 show a dysfunctional phenotype. Interestingly, despite the expression of inhibitory receptors and a lack of signs of immunopathology, intrahepatically primed dysfunctional cells show high expression of effector genes such as granzymes and Ccl5. These effector molecules are released after ex vivo peptide stimulation. Furthermore, the phenotype of intrahepatically primed stem-like and dysfunctional cells only partially overlaps with previously described precursor and terminally exhausted T cells.

Elucidating the mechanism of dysfunctional CD8+ T cell priming has a substantial impact on immunotherapeutic strategies in HBV. Additionally, dysfunctional or exhausted T cells are a hallmark of chronic infections and cancer. Consequently, the insights generated here are broadly applicable.
The emerging role of autoreactive T cells in Guillain-Barré syndrome

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Guillain-Barré syndrome (GBS) is a rare disabling disease that affects the peripheral nervous system (PNS). While evidence from animal studies suggests that pathogenic T lymphocytes targeting PNS-myelin antigens contribute to the disease, the immune-mediated mechanisms underlying the disease in humans are still unknown.

The aim of this study is to investigate the existence and to provide an in-depth characterization of autoreactive T cells in GBS patients during the acute and recovery phases of the disease. To this end, we employed a very sensitive workflow based on ex vivo T cell screenings, generation of single T cell clones and TCR sequencing. Autoreactive memory CD4⁺ T cells targeting PNS-myelin antigens were detected in all GBS patients analyzed so far (n = 10), whereas they were almost absent in healthy controls. Moreover, we have generated and characterized more than 500 autoreactive single T cell clones showing that these cells have a polyclonal TCR repertoire, target multiple epitopes of the self-antigens with some immunodominant regions and are mostly HLA-DR restricted.

Collectively, these findings provide the first solid description of autoreactive T cells directed against PNS myelin antigens in GBS patients, thus further supporting the notion of GBS as an autoimmune disease and opening a new perspective for biomedical application.
Construction and characterization of a panel of immortalized Natural Killer cell lines expressing allelic variants for FCGRIIIA

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Natural Killer cells destroy virally infected and malignant cells by direct cytotoxicity. Antibody-dependent cellular cytotoxicity (ADCC) requires FcγRIIA (CD16) engagement by immunoglobulin G (IgG) on NK cells. Several allelic variants of FCGR3A exist. The high-affinity SNP V158F associates to monoclonal antibody efficacy, whereas SNP L48H/R, shows different binding to IgGs.

NK92 is an IL2-dependent cell line lacking CD16 expression. Our goal was to generate CD16-expressing NK92 cell lines with the most common combinations of the SNPs L48H/R and V158F and test them in ADCC and direct cytotoxicity assays. We cloned FCGR3A into pVITRO and generated variants by site-directed mutagenesis. The cells were electroporated and the expression of CD16 measured by flow cytometry. ADCC consisted of anti-CD20/ anti-EGFR antibodies, Daudi, Raji and A431 as target cells. Additionally, direct cytotoxicity was tested with K562 cells lacking MHC-I.

We produced six NK92 cell lines possessing FCGR3A SNPs combinations (V158F, L48H/R). There was no difference in the lytic activity of NK92 transfectants in response to monoclonal antibodies engaged by CD16, regardless of the genetic variants involved. Despite this, ADCC was dependent on anti-CD20/ anti-EGFR concentrations in the assay. NK92LL_VF showed the highest killing for all E:T ratios tested. NK92RR_VV, on the other hand, had the lowest killing of all transfectants compared to NK92pVITRO and NK92parental controls.

We developed a sustainable ADCC study tool. NK92 transfectants performed ADCC in similar manner, regardless of the SNPs. However, R homozygosity (SNP L48H/R) showed a diminished direct cytotoxicity by NK92 cells, and the mechanisms involved are under examination.
Omicron-Specific Cytotoxic T-Cell Responses After a Third Dose of mRNA COVID-19 Vaccine Among Patients With Multiple Sclerosis Treated With Ocrelizumab

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The SARS-CoV-2 variant Omicron escapes neutralizing antibodies elicited after COVID-19 vaccination, while T-cell responses might be better conserved. It is crucial to assess the effect of the third vaccination, particularly for immunocompromised patients with readily impaired antibody responses.

Twenty adults with multiple sclerosis (MS) under anti-CD20 treatment (ocrelizumab) and receiving a third dose of mRNA COVID-19 vaccine were enrolled in this prospective cohort study conducted at the University Hospital Geneva from March to November 2021. Blood samples were collected at the administration of the third dose and one month later.

Spike-specific CD4 and CD8 T-cell responses against the vaccine strain, and Delta and Omicron variants were maintained in 9 to 12 patients 6 months after the second vaccination, albeit at lower median frequencies against Delta and Omicron variants compared with the vaccine strain (CD8 T cells: Delta, 83.0%; 95% CI, 73.6-114.5; Omicron, 78.9%; 95% CI, 59.4-100.0; CD4 T cells: Delta, 72.2%; 95% CI, 67.4-90.5; Omicron, 62.5%; 95% CI, 51.0-89.0). A third vaccine dose enhanced the number of responders to all variants (11 to 15 patients) and significantly increased the CD8 T-cell responses, but the frequencies of Omicron-specific CD8 T cells remained 71.1% (95% CI, 41.6-96.2) of the vaccine-strain-specific responses.

In conclusion, the data show robust T-cell responses recognizing spike proteins from Delta and Omicron variants, suggesting that COVID-19 vaccination may protect patients on B-cell-depleting drugs against serious complications from COVID-19 infection. T-cell response rates increased after the third dose, demonstrating the importance of a booster dose.

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Identifying factors underpinning the response to PD1-IL2v therapy with a special focus on the role of resource t cells

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PD-1 blockade is the most potent and routinely used standard of care for several cancer types. Its efficacy is limited by factors such as adaptive resistance mechanisms, tumor immune contexture, and intrinsic resistance pathways. IL-2 is used as treatment option for melanoma and renal cell carcinoma. Despite its potency, IL-2 therapy is limited by its toxicity, short half-life, and the activation of Tregs and endothelial cells via IL2Ra binding. PD1-IL2v has been developed to overcome these limitations by combining a mutated IL2v, with abolished binding to CD25 (IL2Rα) to reduce the targeting of immunosuppressive Tregs and endothelial cells, to a high affinity anti-PD1 blocking moiety. Its mechanism of action relies on the simultaneous blockade of PD-1 and IL-2R agonism in cis to PD-1+ tumor-specific CD8 T cells. The aim of this study is to identify factors associated with response to PD1-IL2v therapy using both pre-clinical and clinical data. We would like to dissect the characteristics that distinguish responder from non-responder patients and to understand the composition of the TME (pre and post-treatment) by using mouse tumor models that best reflect human settings. The identification of such biomarkers would allow us to enrich for patients with the highest chances of benefitting from PD1-IL2v therapy and identify potential combination therapies to improve rates and duration of patient responses.
Evaluation of extracellular vesicles as noninvasive early predictive markers for severe COVID-19

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There is an urgent need to identify novel biomarkers to detect as early as possible severe cases of COVID-19 that necessitate urgent and intensive care. Extracellular vesicles (EVs) contain cargoes derived from the cell of origins that include proteins, lipids, and nucleic acids. As a consequence of cellular activation, EV concentration is often increased during disease development and specific EV biomarkers can be found in biofluids.

Here, we propose an innovative approach to identify novel biomarkers for the early detection of severe disease based on EVs. We will collect EVs from broncho-alveolar lavage and plasma of COVID-19 patients during the acute phase. We will correlate the clinical data of patients with the EV molecular characterization at the proteomic and transcriptomic levels. First, we will quantify and characterize EVs in biofluids from COVID-19 patients. Second, we will use a combination of mass spectrometry-based proteomics and transcriptomics to establish a diagnostic model relying on the expression level of multiparameter. Third, the validation of our signature biomarkers will be performed in an independent cohort of patients to control for accuracy (sensitivity and specificity) and the capacity to predict moderate versus severe at the time of diagnosis.

The achievement of these goals will provide deep knowledge about the biological mechanisms that define the different degrees of severity observed in patients with similar characteristics. The identification of the proteins and nucleic acids that normalize their levels after infection will be promising indicators of both the recovery and the evolution of the disease.
Kinase signaling networks defining distinct macrophage phenotypes

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Macrophages are among the most abundant cell types in tumor immune microenvironments characterized with a plethora of phenotypic states, as illustrated with pro-inflammatory M1-like and immunosuppressive M2-like states. M2 macrophages, which are known to assist the process of wound healing, are often hijacked by tumor cells to sustain their proliferation and promote angiogenesis. In contrast to M1 phenotypes, only fragments of signaling networks in the M2 polarization states are currently mapped. In order to address this gap, we performed a global characterization of kinase signaling cascades in primary human macrophages by applying mass spectrometry-based phosphoproteomics. For this, monocytes from the blood of healthy donors were isolated and differentiated into M1(LPS+INF-\gamma), M2a(IL-4+IL-13) and M2c(IL-10) macrophages phenotypes in-vitro. Phosphoproteomics data was used to map the kinase interaction landscape by analyzing patterns in the measured phosphosites and their surrounding sequence motifs, and by inferring the signal flow using both curated and predicted regulatory interactions. Further, we integrated publicly-available transcriptome data to interrogate dominant alternative splicing isoforms of the measured proteins. This suggested that a fraction of the identified kinases, as well as their upstream membrane proteins, and downstream transcription factors, expressed distinct isoforms in different macrophage phenotypes. Among others, the signaling network of M2a macrophages showed that they were characterized with a signal flow through PI3K/AKT/mTOR and RAS/MAPK axes and highlighted PDPK1 kinase as a likely master regulator. Hits identified as central regulators in the signaling networks have a translational potential, as their targeting could drive repolarization from protumoral to anti-tumoral macrophage phenotypes.
Functional analysis of PARP1 in NK cells in the context of inflammation and tumorigenesis

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PARP inhibitors (PARPi) are potent FDA-approved anti-cancer drugs that are highly effective in the treatment of BRCA1/2 mutated cancers, as a consequence of synthetic lethality. Most PARPi target PARP1, a ubiquitous nuclear protein that plays an essential role in regulating the DNA damage response. In addition, PARP1 is an important mediator of the inflammatory response. In vivo administration of PARPi reduces the expression of proinflammatory cytokines after LPS-induced septic shock. Similarly, PARP1 knockout animals exhibit reduced release of the proinflammatory cytokines TNF-\(\alpha\) and IFN-\(\gamma\) and show increased survival after LPS injection. However, the cell types responsible for PARP1-dependent cytokine production and the underlying mechanism remain unknown. Here, we provide evidence that PARP1 is essential for IFN\(\gamma\) production in LPS, and IL18/12 stimulated murine and human NK cells, respectively. In an ectopic colon cancer mouse model, PARP1 knockout in NK cells correlated with increased tumor size, transcriptional down-regulation of NK cell-specific chemokines and a reduction in T cell tumor infiltration. These results suggest that PARP1 regulates cytokine and chemokine expression in activated NK cells and is critical for infiltration of T effector cells, highlighting its novel role in the onset of a potent anti-tumor immune response.
Lipid metabolism remodeling as an anti-inflammatory adaptation to increased chronic translation errors in liver

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ER-Golgi protein secretory pathway is very important for hepatocytes and its perturbation is contributing to the development of chronic liver diseases such as non-alcoholic steatohepatitis (NASH) and hepatocellular carcinoma. We currently lack knowledge of which factors stimulate liver steatosis and we speculate that age-related proteostasis decline may be relevant. We created mice expressing mutant cytosolic ribosomal protein RPS-A226Y which confers cell-nonspecific increased mistranslation and protein misfolding. Next, we did a complex phenotype analysis, including behavioral, histological, and functional genomics studies. Our transcriptomic analysis showed that the liver of 15m-old mutant females has no visible signs of inflammation or activated ER-UPR response achieved by epigenetic reprogramming compared to WT. Here we focus on the metabolic profile and add more molecular details to our understanding of how hepatocytes mitigate chronic ER stress without activating pro-apoptotic UPR. Stress-induced ceramide is well known for triggering cell death. We observe increased ceramide transport from ER into Golgi, transformed to sphingomyelinins, glucosylceramides that accumulate. Additionally, we see an accumulation of secondary bile acid (and chemical chaperone) TUDCA (tauroursodeoxycholic acid) which is produced by gut microbiota from hepatic primary BA. Systemic corticosterone action reduces innate inflammatory pathways most importantly NF-kB and attenuation of FA synthesis avoids toxic lipogenesis. Collectively this orchestrated cellular response illustrates how hepatic cells adapt to proteotoxic stress which commonly increases with age and contributes to chronic diseases.
Longitudinal analysis of SARS-CoV-2 specific memory B cell formation up to 1 year after infection and recall response upon antigen re-exposure

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Immune memory is a hallmark of the adaptive immune response. Germinal center (GC) derived memory B cells can be long lived and are able to rapidly secrete antibodies upon antigen reencounter. Furthermore, they provide an important barrier against viral escape mutants¹. Early pandemic data in severely ill COVID-19 patients showed signs of a strong extrafollicular B cells response, which could hamper the quality of the GC response³. Therefore, an important question relates to the longevity and quality of the B cell response upon severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.

To study the antigen-specific memory B cell formation upon infection we analyzed samples from a longitudinal COVID-19 patient cohort (n=65) using spectral flow cytometry and single-cell RNA sequencing (scRNA-Seq) during the acute disease as well as 6 and 12 months after infection. 38 patients were vaccinated after recovery, allowing us to investigate the recall response.

We find a stable induction of SARS-CoV-2 spike specific memory B cells up to 1 year. The results are comparable in mild and severe COVID-19 patients. Frequencies of specific memory B cells strongly increase after mRNA vaccination. In the acute phase the antigen-specific cells were CD71hi and highly proliferative, after 6 to 12 months the cells acquired a resting memory phenotype which was CD21+ CD27int and mainly expressed IgG1+. Currently, we are characterizing the transcriptional signatures and clonal relations of the different antigen-specific B cell subsets using scRNA-Seq.

Poster 22

A novel universal antibody-based adaptor platform for cancer immunotherapy

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Bispecific immune cell engagers are showing promise in cancer immunotherapy and there is high interest to develop approaches tackling tumor heterogeneity and enabling combination therapies. Here, we present a novel modular universal anti-P329G antibody platform aiming to address those issues. Specifically, for targeting different tumor antigens, primary IgG1 antibodies bearing P329G LALA mutations in the Fc to abolish Fc-immune effector functions are applied. For subsequent effector cell recruitment/activation, secondary antibody-based effector cell engagers directed against the P329G mutation are utilized, including ADCC-competent P329G antibodies (P329G-IgG), P329G-T cell bispecifics (P329G-TCB), or P329G-directed costimulatory antibodies (P329G-CD28/4-1BBL).

In vitro assays showed P329G-TCB induced T cell activation and T cell-mediated tumor cell killing when combined with different tumor-targeted P329G-containing IgG1 antibodies, while no effect was observed in absence of the IgGs. Cytokine release by human PBMCs increased when P329G-CD28, P329G-4-1BBL were combined with the P329G-TCB. These results provide preliminary in vitro evidence that the universal P329G platform can be used as an efficacious cancer treatment. Ultimately, this approach may enable off-the-shelf personalization via combination of tumor targeting antibodies and universal effector cell engagers.
Identification and characterization of lipid-specific unconventional T cells in health and disease

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Unconventional T cells primarily recognizing non-peptide antigens, such as lipids and glycolipids, on monomorphic CD1a, CD1b and CD1c molecules have been described in humans. While their ability to target microbial lipids has been largely described, their involvement in human autoimmunity has been recently proposed. However, due to the lack of sensitive methods to study lipid-specific T cells in humans, this T cell population remains still poorly defined. In this project, we are optimizing a new experimental approach based on the combination of in vitro T cell screenings and ex vivo tetramer staining to identify and characterize human CD1-restricted lipid-reactive T cells from the blood of healthy donors and patients. To this end, we engineered CD1-expressing cell lines to knock out MHC-class II molecules and stably express CD80, CD86 and 4-1 BBL co-stimulatory molecules on their surface. When these newly generated cell lines were used as antigen presenting cells in in-vitro stimulation assays of total CD4⁺ cells from the blood, we were able to identify and isolate CD1-restricted T cells reactive to microbial lipids. In parallel, the staining of peripheral blood mononuclear cells (PBMCs) from healthy donors with CD1 tetramers loaded with the self-glycosphingolipid sulfatide, which is particularly enriched in the myelin of the central nervous system, identified reactivity in different T cell populations with naïve CD4⁺ T cells being the most represented. Overall, this approach will allow us to identify and characterize lipid-reactive T cells in different disease settings, thus potentially shedding light on their so far sparsely described biology, which can be of crucial importance in further understanding general T cell mechanisms in health and autoimmunity.
B and T cells in Autoimmune Hepatitis

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Background and Aims: Autoimmune hepatitis (AIH) is a chronic inflammatory liver disease of unknown aetiology. Antibodies targeting SEPSECS (SLA), which are present in 10-30\% of AIH-I & AIH-II, are highly specific for AIH and associated with more aggressive disease. In order to define the pathogenesis of AIH we investigated B and T cell reactivity to SEPSECS at clonal level.

Method: Blood and liver biopsies were collected from the Swiss Autoimmune Liver Disease Cohort Study and total memory CD4\(^+\)CD45RA\(^-\) T cells and CD19\(^+\) IgG\(^+\) memory B cells were isolated. T cells were labeled with CFSE, incubated with 50 overlapping icosameric peptides spanning the full SEPSECS and proliferation assessed by CFSE dilution. CD25\(^+\) iCOS\(^{high}\) proliferating T cells were cloned by limiting dilution and specificity was assessed by measuring proliferation in response to co-culturing with APCs pulsed with SEPSECS peptides using a \(^3\)H thymidine incorporation assay. B cells were immortalized by infection with Epstein-Barr virus, stimulated by TLR9 activation and cloned by limiting dilution. Supernatants were screened for SEPSECS-specific antibodies and 71 B cell clones isolated, their BCRs sequenced and recombinant antibodies produced.

Results: We identified SEPSECS-specific CD4\(^+\) memory T cells in anti-SLA-positive type 1 AIH, and anti-SLA-negative type 1 and type 2 AIH demonstrating that AIH patients mount a strong CD4\(^+\) T cell response against SEPSECS even in the absence of a humoral response. SLA-positive AIH patients had high numbers of SEPSECS-specific B cells producing high affinity autoantibodies. SEPSECS-reactivity of these antibodies is acquired by mutations in the vdj-region.
GM-CSF polarizes tumor infiltrating myeloid cells to an immune suppressive state

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After immune checkpoint blockade revolutionized cancer therapy, the hunt continues for drugs enabling treatment of immune checkpoint non-responders. Targeting tumor microenvironment dysfunction is gaining momentum as a new therapeutic approach, in particular the reprogramming of suppressive myeloid cells. This project explores that idea by interrogating the functional effect of lymphocyte-secreted granulocyte-macrophage colony-stimulating factor (GM-CSF) on myeloid cells within the tumor microenvironment. We show the sources of GM-CSF in the tumor microenvironment and its impact on tumor growth. Further experiments will uncover GM-CSF’s effect on the various tumor infiltrating myeloid cells.
The role of MMP-12 in MS-associated neuroinflammation

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Matrix Metalloproteinases (MMPs) are often assigned to be involved in the turnover, catabolism and degradation of extracellular matrix. However, in the past few years it has become clearer that MMPs also hold important roles in controlling aspects of inflammation and immunity by regulating the activity of chemokines, cytokines and growth factors. MMP-12 is especially important for the migration of monocytes and macrophages into the inflammatory site of the tissue. It was described to play a detrimental role in several chronic diseases, such as atherosclerosis, where MMP-12 is involved in plaque progression and instability. In experimental autoimmune encephalomyelitis (EAE), a disease model resembling multiple sclerosis, MMP-12 is referred to have opposite effects, as its expression is associated with anti-inflammatory functions. Accordingly, one study showed that MMP-12⁻/⁻ null mice have a poorer EAE outcome compared to their wild-type littermates. Interestingly, our single-cell RNA sequencing datasets revealed that MMP-12 is expressed in CNS invading monocyte derived dendritic cells (moDCs) and macrophages (moMACs) in a GM-CSF-dependent manner. This goes in line with the data Croxford et al. published, where MMP-12 expression is downregulated in CSF2rb¹lacZ/lacZ mice. GM-CSF is a central mediator in MS-associated neuroinflammation and mice lacking GM-CSF in CCR2⁺Ly6Chigh monocytes are resistant to EAE. Thus, we hypothesise that the absence of MMP-12 in monocytes and monocyte derived cells (MdCs) might ameliorate the course of EAE. This is consistent with the explanation that the worse course of MMP-12⁻/⁻ null mice during EAE is due to the importance of MMP-12 for developmental myelination.
Deciphering the immune mechanisms operating in bone metastatic niche

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The bone marrow is not only a main site for hematopoiesis, but also the third most common site of metastasis. The bone metastatic niche is considered as a key element for supporting tumor survival and proliferation, but the contribution of the immune system to its surveillance has not been well studied. In this study, we propose the usage of a recently published innovative immunofluorescent technology by which metastatic cancer cells release a fluorescent protein that penetrates neighboring cells, allowing for labeling and discrimination of the local metastatic microenvironment from the healthy tissue. Using this system, we aim to characterize the immune cell-tumor cell interaction at early and late stages of bone metastasis development. The labeling capacity of the engineered tumor cells has been validated in vitro using co-culture assays using flow cytometry and fluorescent imaging. We also validated our system in vivo using a well-established experimental model of lung metastasis and identified labelling in the lymphoid compartment as well as phagocytosis events in myeloid cells. We now set out to investigate niche-cell labeling in the bone tissue using a recently reported experimental model of bone metastasis where tumor cells are injected into the caudal artery and delivered specifically into the bones. Here, we plan to identify and characterize phenotypically and functionally immune cells in the bone metastatic niche and the corresponding draining lymph nodes. Altogether, the experiments proposed here will identify metastasis-site specific immune cell subsets amenable for therapeutic manipulation.
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100-plex Grant Program
Deep Spatial Phenotyping for “Hallmarks of Cancer”
Application Deadline: June 30, 2022

Turbocharge Your Research with Our Deep Spatial Phenotyping Grant Award

Akoya Biosciences invites scientists involved in immunoncology research to apply for a deep spatial phenotyping “Hallmarks of Cancer” grant award.

The PhenoCycler Fusion system delivers unprecedented speed and depth enabling researchers to scale up unbiased discovery. Combined with the 100-plex “Hallmarks of Cancer” panel, this assay provides deep insights into the eight functional pathways that define the formation of malignant tumors.1

The grant recipient will receive:
- Deep spatial phenotyping data to reveal the presence of 100 cancer biomarkers
- Spatial Insights for up to 3 FFPE tissue samples
- An assay report on results of the PhenoCycler Fusion workflow summarized by Akoya’s application team

References

HOW TO APPLY
Submit a 300-word abstract on how obtaining a deep spatial perspective of your tissue samples at single-cell resolution would further support your immunoncology research projects.

Submissions close on June 30, 2022

“Hallmarks of Cancer” Panel
100 Biomarkers for Deep I/O Spatial Insights

- Avoiding immune destruction
- Tumor promoting inflammation
- Inducing angiogenesis
- Activating invasion and metastasis
- Deregulating cellular energetics
- Sustaining proliferative signaling
- Evading growth suppressors
- Resisting cell death

Unbiased | Ultrahigh-plex
High-Res | High-throughput

Learn more at AKOYABIO.COM/100-PLEX-GRANT or email us at INFO@AKOYABIO.COM for more details.

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100-plex Genomics

SCANNABLE APPLICATION

May 23, 2022 38
REIMAGINE CYTOMETRY

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