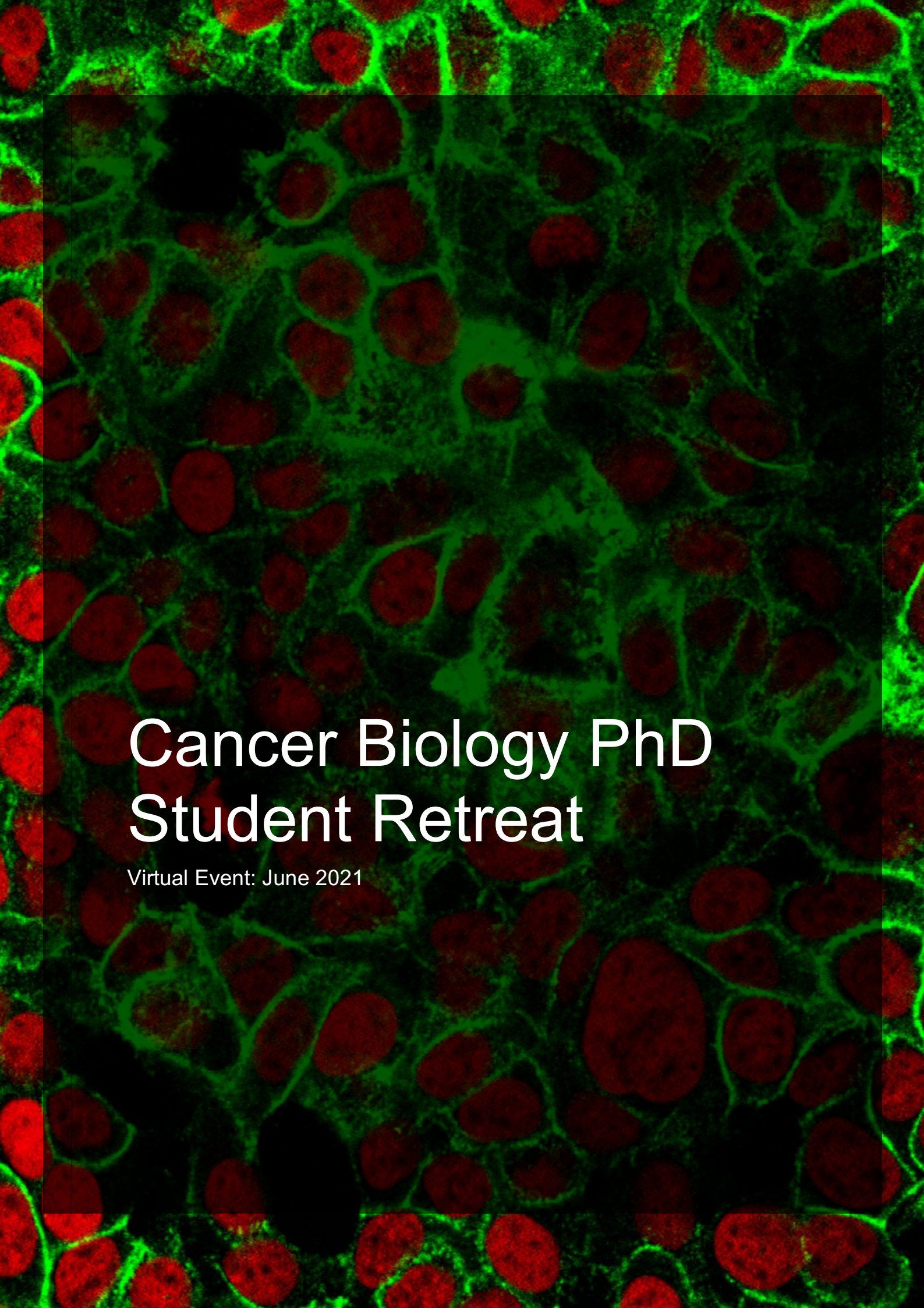


Cancer Biology PhD Student Retreat

Virtual Event: June 2021

8th Cancer Biology PhD Student Retreat

Welcome!

The biennial Cancer Biology PhD Retreat is back, and this time with an upgrade in its 8th edition. Every year, students from the Cancer Biology PhD program at the Life Science Zurich Graduate School meet in the rejuvenating swiss country sides to discuss their research and interact with thought leaders in the field of cancer research. Considering the COVID-19 pandemic, and to ensure you do not miss the opportunity to engage with your peers, the retreat has been shifted to a virtual format. There is an exciting line-up of keynote lectures and a panel discussion, with experts sharing their insights on different topics of cancer biology and on how to take a good start in your future career after the PhD.

Organizing committee

Some of your colleagues were happy to help put together this virtual retreat. Here is the organizing committee of 2021.

Devmini Moonamale
Sarcoma group, Oncology Research Department, University Children's Hospital Zürich

Harini Lakshminarayanan
Department of Pathology and Molecular Pathology, University Hospital Zurich, and University of Zurich

Milica Zecevic
Pediatric Molecular Neuro-Oncology Research, University Children's Hospital Zürich

Roxanne Lourman
Laboratory of Molecular Neuro-oncology, Department of Neurology, University Hospital Zurich

Sija Sajibu
Institute of Molecular Cancer Research (IMCR), University of Zurich

Bettina Rausch Malina
Program Coordinator Cancer Biology Ph.D. Program

Maries van den Broek
Program Director Cancer Biology Ph.D. Program

Speakers

Prof. Dr. Nicola Aceto
Institute of Molecular Health Sciences
ETH Zurich
Nicola.aceto@biol.ethz.ch

Dr. Dominik Schelshorn
Campus Biotech
Addex Therapeutics
dominik.schelshorn@addextherapeutics.com

Dr. Jennifer Spangle
Winship Cancer Institute
Emory University
jennifer.spangle@emory.edu

Dr. Vladimir Cmiljanovic
Swiss Rockets, Basel
vladimir.cmiljanovic@swissrockets.com

Prof. Dr. Isabelle Arnold
Institute of Experimental Immunology
University of Zurich
arnold@immunology.uzh.ch

Assoc. Prof. Dr. Ellen Heitzer
Institute of Human Genetics
Medical University of Graz
Ellen.heitzer@medunigraz.at

Program

02 JUNE 2021

9:00 AM - 9:15 AM
Welcome Address: Prof. Dr. Maries van den Broek

Welcome!
Please follow this zoom link to join us at the retreat:
https://uzh.zoom.us/j/64638726907?pwd=M0F6Z293amMrYzBDcnUxOG94MFYzZz09
Meeting ID: 646 3872 6907
Passcode: 0203062021

9:15 AM - 10:00 AM
Session 1: Prof. Dr. Nicola Aceto
ETH Zurich, Department of Biology, Molecular Oncology (Zurich, Switzerland).
The Aceto Lab aims to gain fundamental insights into the biology of CTC-clusters, and to identify novel therapeutic targets to suppress the metastatic spread of cancer.

10:00 AM - 10:20 AM
CD39+PD-1+CD8+ T cells mediate metastatic dormancy in breast cancer
Héctor Castañón
Institute of Experimental Immunology, UZH

10:20 AM - 10:40 AM
Quantification of Protein Degradation Rate Constants in the Living Cell
David Vukovic
Department of Biochemistry, UZH

10:40 AM - 11:00 AM
Break

11:00 AM - 11:20 AM
Single-cell profiling reveals a conserved myogenic hierarchy in pediatric rhabdomyosarcomas associated with drug resistance
Sara Danielli
Division of Oncology, University Children's Hospital Zurich

11:20 AM - 11:40 AM
Endogenous Retrovirus expression activates type-I interferon signaling in an experimental mouse model of mesothelioma development
Suna Sun
Department of Thoracic Surgery, USZ

11:40 AM - 1:30 PM
Poster session 01 + Lunch
Presentations (8)

MSH6 depletion promotes tumor growth in a RCAS/tv-a glioblastoma mouse model - Roxanne Lourman

Radiotherapy treatment volume in a mouse model of radiation-induced lymphopenia - Irma Telarovic

The molecular landscape of proteasome inhibitor resistance in multiple myeloma- Jonas Schwestermann

CanIsoNet v1.0: Database to dissect the functional impact of Isoform Switching Events in Cancer - Tülay Karakulak

Unraveling a novel concept of fractionation radiotherapy for the treatment of solid tumors - Irene Vetrugno

New Methodology for Antibody Discovery and Engineering - Julian Weischedel

Integrative overview of molecular mechanisms driving non-hodgking lymphomagenesis in HIV/EBV infected individuals - Karla Cervantes

The neural crest stem cell marker NGFR confers immune evasion to metastatic melanoma cells - Julia Lehmann

1:30 PM - 2:15 PM
Session 2: Prof. Dr. Jennifer Spangle
Winship Cancer Institute of Emory University, Department of Radiation Oncology, Division of Cancer Biology (Atlanta, Georgia, USA).
The Spangle Laboratory is committed to understanding how PI3K-mediated regulation of the epigenome contributes to cancer development and modulates treatment response.

2:15 PM - 2:35 PM
The Hypoxic Response in Natural Killer Cell- Mediated Tumour Surveillance
Ekaterina Khatchatourova
Institute of Anatomy, UZH

2:35 PM - 2:55 PM
Exploring the immune response after locoregional treatment of peritoneal metastasis
Lilian Roth
Department of Surgery and Transplantation, USZ

2:55 PM - 3:15 PM
Senataxin preserves genome integrity by counteracting R-loop accumulation
Satyajeet Rao
Institute of Molecular Cancer Research, UZH

3:15 PM - 3:30 PM
Break

3:30 PM - 3:50 PM
A novel niche-perturbation model to study the role of secreted Wnt ligands in colorectal cancer
Michael Brügger
Institute of Molecular Life Science, UZH

3:50 PM - 4:10 PM
The landscape and dynamics of eosinophil diversity and plasticity in colorectal cancer
Alessandra Gurtner
Institute of Experimental Immunology, UZH

4:10 PM - 5:30 PM
Poster Session 02
Presentations (10)

Functional analysis of ARTD1 in NK cells in the context of inflammation and tumorigenesis - Flurina Böhi

Developing a noninvasive biomarker assay for early detection of colorectal cancer - Sija Sajibu

Development of novel local treatments for peritoneal metastasis - Linda Russo

Extracellular vesicles influence B cell maturation signaling - Kevin Yim

Perioperative immunotherapy to control tumor growth in the regenerating liver - Laura Heeb

Combined treatment modalities to overcome hypoxia driven radioresistance - Claire Beckers

Mass Spectrometry-empowered Spatial Single-Cell Proteomics Techniques in Cancer Research - Mengze Zhang

Deciphering the influence of cancer-cell-intrinsic cGAS expression on the tumor microenvironment - Michael Herbst

Characterization and induction of tertiary lymphoid structures in the context of tumor immunity - Anna Laura Calvanese

The role of gene editors in B-cell lymphomagenesis during chronic viral infection - Anna Gramalla-Schmitz

03 JUNE 2021

9:00 AM - 10:30 AM
Panel discussion - Career building

Speakers:
• Dr. Vladimir Cmiljanovic
• Dr. Dominik Schelshorn
• Prof. Dr. Isabelle Arnold

10:30 AM - 10:40 AM
Break

10:40 AM - 11:00 AM
Integrins as targets for CAR T cell therapy for glioblastoma
Danielle Villars
Laboratory of Molecular Neuro-Oncology, USZ

11:00 AM - 11:20 AM
Mechanisms of tumor recurrence and drug resistance in rhabdomyosarcoma
Devmini Moonamale
Division of Oncology, University Children's Hospital Zurich

11:20 AM - 11:40 AM
Nuclear actin polymerization dictates the immediate cellular response to DNA replication stress
Maria Dilia Palumbieri
Institute of Molecular Cancer Research, UZH

11:40 AM - 1:30 PM
Poster session 03 + Lunch

Presentations (9)

The proteomic landscape of primary versus recurrent glioblastoma
- Marcel Bühler

Activation of patient tumor-associated dendritic cells by a combination of Toll-like receptor 8 agonists - Mi He

Impact of Ionizing Radiation on the Energy Metabolism of Healthy and Tumor Cells in the Brain - Marvin Kreuzer

RNA Binding Motif Protein 8A: a novel RNA editing target in mesothelioma
- Martin Wipplinger

Spermidine ameliorates colitis and induces anti-inflammatory macrophages
- Anna Niechcial

The effect of TET2 mutation on phagocytic potential of macrophages and its implications for macrophage checkpoint inhibition - Lisa Dietsche

The immune regulation of liver metastasis - Marc Nater

Modulation of Intracellular Protein Degradation - Dorothea Winkelvoss

Unravelling the mechanisms of immunomodulation in breast cancer
- Tatjana Schmitz

1:30 PM - 2:15 PM
Session 3: Prof. Dr. Ellen Heitzer

"Current and future perspectives of circulating tumor DNA"
Medical University of Graz, Institute of Human Genetics (Graz, Austria).
The Heitzer group investigates the value of liquid biopsies for personalized cancer treatment.

2:15 PM - 2:35 PM
ADAM17-dependent paracrine and intercellular communication in response to irradiation
Fabienne Tschanz
Laboratory of applied Radiology, USZ

2:35 PM - 2:55 PM
Loss of RNA editing enzyme ADAR2 results in growth inhibition and increased chemosensitivity in mesothelioma
Ananya Hariharan
Laboratory of Molecular Oncology, Department of Thoracic Surgery, USZ

2:55 PM - 3:15 PM
Dual role of tumor cell-derived IL-10 in promoting cell-autonomous growth and immune escape in diffuse large B-cell lymphoma
Kristin Stirm
Institute of Molecular Cancer Research, UZH

3:15 PM - 3:30 PM
Closing Remarks

4:30 PM - 8:30 PM
Networking Apero / BBQ

On-site in-person Apero/BBQ. Join us and enjoy the inspiring company of your colleagues over some drinks and food.

Irchel Park

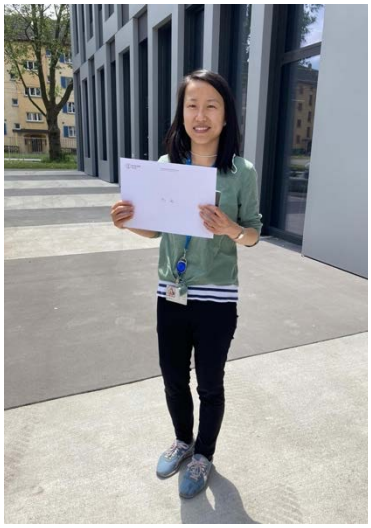
Winners

Poster Prize Winners

Roxanne Lourman
Julia Lehmann
Linda Russo
Michael Herbst
Mi He
Dorothea Winkelvoss

Oral Presentation Winners

Michael Brügger
Maria Dilia Palumbieri



Combined treatment modalities to overcome hypoxia driven radioresistance

Claire Beckers[1], Lazaros Vasilikos[1], Martin Pruschy[1]

[1]Laboratory for Applied Radiobiology, Department of Radiation Oncology, USZ

Hypoxic tumor cells are up to a three-fold more resistant to ionizing radiation than well-oxygenated cells. This resistance is most challenging with treatment regimens using high doses of radiation/fraction (e.g., hypofractionated radiotherapy). Combining hypofractionated radiotherapy with agents that sensitize these resistant tumor cells to ionizing radiation is an interesting approach to overcome this shortcoming. The activation of hypoxic survival pathways plays an important role in acquiring this resistance. Therefore, inhibiting key regulators within the pathways and thereby exploiting their survival mechanisms, gives rise to new potential drug targets for combined treatment modalities with hypofractionated radiotherapy.

We are investigating these survival pathways that are upregulated under hypoxia and that drive radioresistance. By screening for upregulated kinases, new drug targets for combined treatment modalities with radiotherapy might be identified. TOLREMO therapeutics, established in 2017 as a spin-off of ETH Zurich, has already identified the survival kinase DYRK1B (dual specificity tyrosine phosphorylation-regulated kinase 1B) to play an important role in tumor cell survival pathways upregulated under stress factors such as hypoxia and nutrient deprivation. As part of my thesis, we will also investigate the role of DYRK1B in the treatment response to irradiation and its inhibition (using small molecular DYRK1B-inhibitors) in combination with radiotherapy *in vitro* and *in vivo*. With such a combined treatment modality, we aim to overcome the major treatment resistance hurdle of tumor hypoxia in a biologically cooperative approach.

Functional analysis of ARTD1 in NK cells in the context of inflammation and tumorigenesis

Flurina Böhi[1], Michael O. Hottiger [1]

[1]Department of Molecular Mechanisms of Disease, University of Zürich, Switzerland

ARTD1 (PARP1) is an ADP-ribosyltransferase that covalently attaches ADP-ribose to a target protein using NAD+ as substrate. It is best known for its role in DNA damage repair response following single strand breaks. Inhibition of ARTD1 by PARP inhibitors (PARPi) is currently used in clinics to treat *BRCA1/2*-deficient ovarian and breast cancer patients. *BRCA1/2*-mutated cells are deficient in homologous recombination (HR) and thus depend on alternative repair pathways. This vulnerability of HR-deficient tumor cells is exploited by treatment with PARPi in the context of synthetic lethality.Interestingly, besides its function in DNA repair, ARTD1 is also involved in the inflammatory response. Treatment with PARPi reduces the expression of proinflammatory cytokines including IFN-γ after LPS induced systemic inflammation. Similarly, ARTD1 knockout animals show an improved survival rate and a decrease in the release of proinflammatory cytokines TNF-α and IFN-γ after LPS injection. The cell type responsible for the dampening effect of PARPi on pro-inflammatory cytokine expression, particularly IFN-γ, was so far not identified. Due to interesting preliminary results in NK cells we are examining a potential direct effect of PARPi on NK cell activation and cytokine production. We are thus aiming to investigate the cellular signalling pathway(s) regulated by PARPi in activated NK cells and to identify the cellular targets of ADP-ribosylation in NK cells. Moreover, we study the role of ADP-ribosylation and ARTD1 in NK cells in *in vivo* models of cancer and inflammation. Preliminary data will be discussed at the meeting.

A novel niche-perturbation model to study the role of secreted Wnt ligands in colorectal cancer

Michael Brügger[1]

[1]University of Zürich, Department of Molecular Life Sciences

The gastrointestinal epithelium is one of the most rapidly self-replenishing tissues in mammals. The rapid turnover of the entire intestinal epithelium is driven by intestinal stem cells located at the bottom of the crypts in the small intestine and colon. Wnt/B-catenin signalling is important for the self-renewal of the intestinal stem cells. Aberrant ectopic activation of the Wnt/B-catenin signalling pathway (e.g. loss of function of APC) has been implicated in the initiation and progression of colon cancer and affords the cancer cells independence of upstream Wnt ligand input. Regardless, multiple reports attribute an increasing importance to secreted Wnt ligands or secreted Wnt signaling modulators (e.g. Sfrp1) in colorectal cancer progression.In order to study the role of secreted Wnt ligands in colorectal cancer progression and metastasis we are developing a novel niche-perturbation model. In order to perturb the tumour microenvironment in a specific and temporally controlled manner we combine three state-of-the-art techniques: 1. Colonic organoids harbouring tumorigenic mutations (e.g. APC K.O., KrasG12D, Tp53 K.O., Smad4 K.O.) 2. Orthotopic endoscopy-guided injection 3. Cell-penetrating peptides.

In conclusion, this model will allow us to ask novel questions about the effects of perturbations to the tumour microenvironment, by circumventing the oftentimes deleterious effects on mouse health of changes in the whole mesenchyme. Ultimately this model could be easily adapted to a variety of transplantation-based tumour models and therefore pose as a promising tool to the field of tumour microenvironment research.

The proteomic landscape of primary versus recurrent glioblastoma

Marcel Bühler[1]

[1]Laboratory of Molecular Neuro-Oncology, University Hospital Zurich, and University of Zurich

Glioblastoma is the most common and most aggressive primary brain tumour in adults. Treatment options as well as therapy responses in patients are very limited, which ultimately results in fatal disease outcome with a median life expectancy in the range of 16 months. Despite extensive research on the genomic and transcriptomic level, these efforts have not yet been able to identify better therapeutic approaches than the current standard of care, leaving temozolomide-based radiochemotherapy as the standard treatment for newly diagnosed glioblastoma patients and CCNU/Lomustine-based chemotherapy as a “gold standard” for patients with recurrent tumours. Therefore, novel approaches for potential therapeutic target discovery are needed. In contrast to genome-based studies, comprehensive investigations on a functionally relevant protein level are scarce. Pressure cycling technology (PCT) coupled with sequential window acquisition of all theoretical fragment ion spectra (SWATH) mass spectrometry allows fast and reproducible proteomic profiling of limited clinical sample specimens at high sensitivity. We applied PCT-SWATH mass spectrometry to FFPE tissue samples from two independent patient cohorts of matched paired newly diagnosed and recurrent glioblastoma. In both cohorts, the ubiquitin ligase F-box only protein 2 (FBXO2) was more abundant in recurrent tumours. CRISPR/Cas9-mediated knockout of *FBXO2* in human glioma cells resulted in improved survival in orthotopic xenograft mouse models compared to wild-type control cells. In contrast, we did not observe growth differences of *FBXO2* knockout cell lines compared to wild-type controls *in vitro*. These findings point towards a microenvironment-dependent role of FBXO2 in the regulation of tumour growth.

Characterization and induction of tertiary lymphoid structures in the context of tumor immunity

Anna Laura Calvanese[1], Virginia Cecconi[1], Maries van den Broek[1], Karina Silina[1]

[1]Institute of Experimental Immunology, University of Zurich, Winterthurerstrasse 190, 8057, Zurich, Switzerland. Comprehensive Cancer Center Zurich, Zurich, Switzerland

Tertiary lymphoid structures (TLS) develop in non-lymphoid tissues upon chronic inflammation and cancer. They are organized structures that resemble follicles of secondary lymphoid organs in function and architecture. TLS density often correlates with favorable prognosis in different solid cancer types. However, it is not known which cell types, chemokines and cytokines drive the development of TLS. Also, no evidence of the direct role of TLS in anti-tumor immunity has been shown due to the lack of suitable models.

We established a mouse model for TLS induction in the lungs based on intranasal administration of multiple inflammatory stimuli plus an antigen. We found that TLS maturation is a stepwise process that recapitulates different TLS maturation stages seen in human cancer. Using adoptively transferred, T-cell receptor transgenic, ovalbumin-specific T cells, we found that T cells persist in TLS induced by cognate (ovalbumin) as well as non-cognate (NP-KLH) antigens. Using CXCL13-reported mice, we saw that the chemokine CXCL13 is produced very early during TLS development and is likely an essential upstream event.

By combining multiparametric flow cytometry and single-cell RNA sequencing we now aim to dissect the identity of CXCL13-producing cells over the course of TLS development, as well as to identify indispensable cell types and chemokines for TLS development and maturation. Our results will lead to a better understanding of the biology of cancer-associated TLS.

CD39+PD-1+CD8+ T cells mediate metastatic dormancy in breast cancer

Héctor Castañón[1], Paulino Tallón de Lara[1], Marijne Vermeer[1], Nicolás Núñez[1], Virginia Cecconi[1], Karina Silina[1], Joaquín Urdínez[2], Farkhondeh Movahedian Attar[1], Isabelle Glarner[1], Bettina Sobottka-Brillout[3], Holger Moch[3], Sonia Tugues[1], Burkhard Becher[1], Maries van den Broek[1]

[1]Institute of Experimental Immunology, University of Zürich, [2]Department of Orthopaedics, Balgrist University Hospital, University of Zurich Zurich, Switzerland, [3]Institute of Pathology and Molecular Pathology, University Hospital Zurich, Switzerland

Metastasis is the most common cause of breast cancer-related mortality. Some tumors metastasize aggressively whereas others remain in a state of metastatic dormancy for years. The mechanisms governing metastatic dormancy remain largely unknown. Because >60% of breast cancer-related deaths occur >5 years after resection of the primary tumor, understanding mechanisms underlying dormancy is of high clinical relevance.

To investigate the processes mediating dormancy, we used two breast cancer cell lines originally derived from the same spontaneous tumor in immunocompetent BALB/c mice, 4T1 and 4T07. We detected disseminated tumor cells (DTCs) in the blood and lungs of 4T1 and 4T07 tumor-bearing mice. However, only orthotopic 4T1 tumors lead to progressive metastasis in the lungs, whereas 4T07-DTCs persisted as non-proliferating, single cells.

Using genetically modified mice and depleting antibodies, we found that 4T07-dormancy depends on CD8+ T-cells. Through high-parametric flow cytometry, we identified a population of CD39+PD-1+CD8+ T-cells in 4T07 primary tumors and in the lungs with dormant disseminated 4T07 cells, which was hardly found in aggressively metastasizing tumors. Of note, adoptive transfer of purified CD39+PD-1+CD8+ T-cells prevented metastatic outgrowth. Antibody-mediated blocking of IFNg and TNFa in 4T07 tumor-bearing mice precluded dormancy, allowing metastatic outbreaks in the lungs. In human breast cancer, the frequency of CD39+PD-1+CD8+ but not total CD8+ T-cells correlated with increased disease-free survival after tumor resection, underlining the biological relevance of CD39+PD-1+CD8+ T-cells breast cancer metastatic dormancy. Thus, we discovered that a primary breast tumor primes a systemic, CD8+ T-cell response that mediates metastatic dormancy via IFNg and TNFa.

Integrative overview of molecular mechanisms driving Non-Hodgking Lymphomagenesis in HIV/EBV infected individuals

Karla Cervantes[1]

[1]Institute of Experimental Immunology, University of Zurich

Despite the high efficiency of combined antiretroviral therapy (cART) to suppress the progression of the human immunodeficiency virus (HIV) into acquired immunodeficiency syndrome (AIDS), and to decrease mortality and incidence of AIDS-defining cancers, the increased life expectancy of HIV+ individuals through cART has also increased the occurrence and development of these malignancies. Thus, individuals with AIDS are still at high risk to develop cancer, being this the third-leading cause of death in people infected with the virus. Non-Hodgkin Lymphomas (NHLs) are among the most common AIDS-defining cancers and they remain a major cause of morbidity and mortality even after the cART era. Diffuse large B-cell lymphoma (DLBCL) and Burkitt lymphoma (BL) are the most common AIDS-NHL subtypes. They have shown higher incidence and aggressiveness in HIV+ individuals than in the non-infected population. Moreover, coinfection with oncoviruses, such as EBV, has also been associated with NHLs in the HIV+ population. Consequently, there is a major need to elucidate the drivers and mechanisms involved in AIDS-NHL pathophysiology. Traditional hypothesis-driven approaches have provided new insights about the disturbed mechanisms in AIDS-NHL, however, many loose ends remain and are impossible to be described by this approach solely. Therefore, this project aims to provide insights into hidden patterns of the disease through a hypothesis-free systems methodology. It relies on large-scale data analysis of published data. Hence, the main goal is to apply a hypothesis-free approach to identify consistencies among published omics studies, network interactions, and produce unbiased and novel testable hypotheses as a result.

Single-cell profiling reveals a conserved myogenic hierarchy in pediatric rhabdomyosarcomas associated with drug resistance

Sara Danielli[1], Ermelinda Porpiglia[2], Andrea De Micheli[3], Joana Marques[1], Marco Wachtel[1], Beat Schäfer[1]

[1]University Children's Hospital of Zurich, Department of Oncology, Balgrist Campus, Lengghalde 5, 8008 Zürich, Switzerland, [2]Stanford University School of Medicine, Stanford, CA 94305, USA, [3]Institute for Translational Medicine, Department of Health Sciences and Technology, ETH Zürich, 8092 Zürich, Switzerland

Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in pediatric patients. Despite improvements in the cure rates over the last 30 years, patients with the alveolar subtype are still largely considered uncurable, with 5-year overall survival rates below 40%. These patients often develop resistance towards therapy, limiting therapeutic success and making the clinical course of relapsed RMS very poor. One of the emerging determinants of treatment failure in cancer is intratumoral heterogeneity (ITH), a yet unexplored area in RMS. In this project, we propose to characterize ITH in RMS and to decipher its involvement in drug-resistance. Using a combined approach of single-cell RNA sequencing and mass cytometry, we profiled several thousand individual cells from RMS patient-derived xenografts and cell lines and discovered that RMS tumors are composed of heterogeneous cell populations. These populations recapitulate a branched myogenic trajectory, whereby progenitor stem-like cells (SC-like) either commit to differentiation or to actively cycling myoblasts. We show that SC-like cells are characterized by yet undescribed markers, which get upregulated upon exposure to chemotherapeutics. Similarly, markers of committed populations decrease after drug treatment, suggesting that chemotherapeutics shift cellular composition of RMS towards undifferentiated clusters. Current studies are ongoing to determine pathways underlying drug resistance in SC-like cells and ways to pharmacologically interfere with it. Taken together, this study suggests a possible origin for RMS and has the potential to improve the clinical outcome of RMS patients by co-targeting drug-sensitive and drug-resistant subpopulations.

The effect of TET2 mutation on phagocytic potential of macrophages and its implications for macrophage checkpoint inhibition

Lisa Dietsche[1], PD Dr. med. Alexandre Theocharides[1]

[1]University of Zürich

The methylcytosine dioxygenase *TET2* is a DNA modifying enzyme involved in epigentic gene expression regulation recurrently mutated in hematological malignancies such as AML, MDS and MPNs. In hematopoiesis TET2 regulates stem-cell self-renewal1, lineage commitment and differentiation2,3,4. Cytokine expression, immune crosstalk and differentiation of immune cells are also affected by *TET2* mutations, which explains the role of TET2 in anti-tumor immunity and cancer immunotherapy. Interestingly, melanoma progression was reduced in mice with TET2 deficient macrophages causing increased T-cell infiltration7.

One of the possibilities to prevent immune escape is to block the “don’t eat me signal” CD47 expressed on cancer cells. CD47 binds to the macrophage receptor SIRPα, which inhibits phagocytosis. Consequently, blocking the interaction between CD47 and SIRPα induces phagocytosis of cancer cells and reduces tumor growth8-11.

TET2 mutation in macrophages causes a switch in polarization from M2 to the pro-inflammatory M1-type6,7, including increased expression of IL-1β, IL-6 and Arg15. Here we aimed to assess the effect on phagocytic function of *TET2* mutated macrophages and its impact on macrophage checkpoint inhibition. Literature described M1-macrophages to be less efficient in phagocytosis than M2 macrophages12,13. However, the results of a cancer cell phagocytosis assay with *TET2* mutated bone marrow derived macrophages show the opposite. Initial results suggest a phagocytosis promoting effect of *TET2* mutation compared to WT. If this effect is exploitable in macrophage checkpoint blockade such a treatment could be especially beneficial for hematologic cancer or clonal hematopoiesis patients with *TET2* mutation.

The role of gene editors in B-cell lymphomagenesis during chronic viral infection

Anna Gramalla-Schmitz[1], Agshin Balayev[1], Lisa Rieble[1], Karla Cervantes-Gracia[1], Christian Münz[1], Richard Chahwan[1]

[1]Institute of Experimental Immunology, UZH

Human-immunodeficiency-virus (HIV) patients have a higher risk for B-cell lymphomas compared to the general population. One main factor is the reactivation of oncogenic opportunistic herpesviruses: Epstein-Barr virus (EBV) and Kaposi-sarcoma herpesvirus (KSHV). Both viruses infect B cells and are known to be involved in B-cell lymphomagenesis. Furthermore, HIV itself contributes to the transformation of B cells, likely through HIV Tat protein, which is found in the serum of HIV carriers and can enter and alter almost any cell type. How these different viruses individually and additive lead to B-cell lymphoma remains to be elucidated. In the context of an immune response, viruses activate the expression of cellular DNA and RNA editors like the AID/APOBEC and ADAR deaminase families. It was shown that these deaminases are also highly expressed in cancer tissue and their mutation signatures are even more abundant in certain virus-derived cancer. We postulate that chronic viral infections promote mis-regulation of cellular mutators, which leads to multiple off-target mutations in the host genome and finally to B-cell lymphomagenesis.

The landscape and dynamics of eosinophil diversity and plasticity in colorectal cancer

Alessandra Gurtner[1], Costanza Borrelli[2], Andreas Moor[2], Isabelle Arnold[1]

[1]Institutes of Experimental Immunology, University of Zurich, Zurich, Switzerland, [2]Department of Biosystems Science and Engineering, ETH Zurich, Basel, Switzerland

Eosinophils are an integral part of the resident intestinal immune system, and are classically associated with immune effector functions in allergic diseases and protection against parasites. They have also been shown to infiltrate multiple tumors, where, depending on the site of tumorigenesis, they can display both pro- and anti-tumorigenic properties. Indeed, we demonstrated that, in a genetic model of colorectal cancer (CRC), eosinophils presence inversely correlates with tumor burden in the colon, but not in the small intestine. These observations suggest that eosinophils in different organs may be specifically primed by tissue-derived cues, and may thus exhibit distinct functions. However, to date, eosinophil functional heterogeneity and plasticity during inflammation and cancer, as well as in healthy tissues, remains unexplored. To address this gap in knowledge, we performed transcriptomic and high-dimensional flow cytometry analyses of eosinophils isolated from different organs. We show that the gastrointestinal tract is populated by phenotypically distinct eosinophil subsets, and define signature genes and surface markers for their identification.

Our data indicate that eosinophils are differentially primed in the distinct tissue environments, and may thus differently respond to local cancer. These findings may facilitate the development of new therapeutic strategies to unleash the anti-cancer activity of eosinophils in cancer patients.

Loss of RNA editing enzyme ADAR2 results in growth inhibition and increased chemosensitivity in mesothelioma

Ananya Hariharan[1], Manuel Ronner[1], Marika Sculco[1], Jelena Kresoja-Rakic[1], Lucia Oton-Gonzalez[1], Emanuela Felley-Bosco[1]

[1]Laboratory of Molecular Oncology, Department of Thoracic Surgery, University Hospital Zurich, Zurich, Switzerland

A-to-I double-stranded RNA (dsRNA) editing and the expression of RNA editing enzyme, adenosine deaminase acting on dsRNA, Adar2 increased upon asbestos exposure in a mouse mesothelioma model. In addition, high ADAR2 expression is associated with worst overall survival in the mesothelioma TCGA dataset. To elucidate the role of ADAR2 in mesothelioma, we developed ADAR2 knockout cell lines from mouse and human cells using the CRISPR/Cas9 system. ADAR2 knockout cells had reduced COPA (an ADAR2-specific editing target) editing, indicating decreased ADAR2 activity. We observed reduced proliferation of ADAR2 knockout cells in colony forming and spheroid building assays. Cell cycle analysis showed that upon ADAR2 knockout the number of cells entering S-phase reduced with an increase in cells arresting in the G2/M phase. We also documented an increase in expression of autophagy marker, LC3-II. Pemetrexed is a first-line chemotherapy used for mesothelioma patients, and we found that ADAR2 knockout cells were more sensitive to pemetrexed treatment. Dihydrofolate reductase or DHFR, is a target of pemetrexed, whose expression is regulated by ADAR-induced A-to-I editing. We found that ADAR2 knockout results in reduced DHFR expression. These observations suggest that ADAR2 contributes to increased cell growth in mesothelioma possibly by downregulating autophagy. ADAR2 may also play a role in the resistance towards pemetrexed in mesothelioma via the upregulation of DHFR expression controlled by A-to-I RNA editing of the DHFR transcript.

Superior human dendritic cell activation by Toll-like receptor 8 agonist combinations

Mi He[1]

[1]Roche Innovation Center Zurich

Dendritic cells (DCs) are professional antigen presenting cells crucial for the induction of immune response to pathogens and tumor cells. Both DC signature and DC activation in the tumor correlate with improved patient survival. However, dendritic cell numbers are low and there is evidence that the tumor microenvironment suppresses DC functions. Exogenous signals, such as pattern recognition receptor agonists and interferons can promote DC recruitment to the tumor, DC maturation and improve DC functions. Currently, it is unclear which stimulation allows best activation and recruitment of human DCs in the tumor microenvironment. We hypothesized combinatorial stimulation of DCs will lead to superior DC activation and better anti-tumor responses in human tumors. The goal of my PhD project is to determine the optimal treatment for human DC activation and understand the underlying mechanisms of the combination benefits in order to induce a strong and long lasting anti-tumor immune response.

Different combinations of pattern recognition receptor agonists and interferons were tested for their ability to induce the secretion of IL-12p70, a potent Th1 cytokine, in human DCs. Toll-like receptor 8 agonist combinations synergize in the expression of IL-12p70 and many other cytokines in human cord blood and blood DCs. The observed synergistic effects were combination specific, which presumably will result in the support of different DC functions. Benefits of combinatorial stimulation were further validated in DCs from patient tumor digests. In conclusion, Toll-like receptor 8 agonist combinations are promising candidates for the activation of tumor DCs in future cancer immunotherapies.

Perioperative immunotherapy to control tumor growth in the regenerating liver

Laura Heeb[1]

[1]Universitätsspital Zürich

The liver has a remarkable capacity to regenerate and up to two thirds can be safely removed. Therefore, the gold standard to treat liver cancer is the surgical resection of malignant tissue, called hepatectomy. However, if the future liver remnant is too small, liver regeneration is impaired and consequently leads to liver failure, thus limiting the success rate at very progressed cancer stages.

Serotonin has been shown to aim liver regeneration. On the other hand, our recent study shows that the presence of serotonin leads to the upregulation of PD-L1 in the tumor microenvironment, thereby inhibiting the CD8+ T cell response against the tumor, leading to enhanced tumor growth. My project aims at elucidating the mechanism of how serotonin influences the tumor microenvironment. My *in vitro* experiments suggest that PD-L1 upregulation on cancer cells in the presence of serotonin is due to histone serotonylation, a direct modification of histones by serotonin, which leads to epigenetic changes.

Furthermore, during liver regeneration, micrometastatic lesions that could not be resected start to grow again, leading to cancer relapse. This is probably due to the growth-inducing microenvironment in the regenerating liver. By using immunotherapy during the perioperative window, I aim to prevent cancer relapse during liver regeneration. For this, we developed a mouse model to mimick the liver after tumor resection and aim to treat these mice with antibodies against immunomodulatory molecules such as PD-L1, PD-1 and LAG-3 to efficiently eradicate remaining micrometastatic lesions, allowing the liver to safely regenerate after hepatectomy.

Deciphering the influence of cancer-cell-intrinsic cGAS expression on the tumor microenvironment

Michael Herbst[1, 2], Silvan Brunn[1, 2], Virginia Cecconi[1, 2], Maries van den Broek[1, 2]

[1]Comprehensive Cancer Center Zurich, Switzerland, [2]Institute of Experimental Immunology, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland

Sensing DNA by cyclic GMP-AMP (cGAMP)-synthase (cGAS) results in production of cGAMP-dinucleotide, consecutive activation of stimulator of interferon genes (STING) and subsequent production of type I interferon (IFN-I). Because cancer cells contain elevated amounts of cytoplasmic DNA, cGAS-STING-signaling is particularly relevant in the context of tumor immunology. We previously showed that cancer-cell-intrinsic expression of cGAS leads to IFN-I-dependent tumor-infiltration by CD8+ T-cells, resulting in better tumor control (Schadt L et al., Cell Reports 2019).

We now aim to better understand how local IFN-I production influences the tumor microenvironment (TME). First, using Mx1GFP-reporter mice (Uccellini M B & García-Sastre, Cell Reports 2018), we will investigate which cell types respond to IFN-I over time in the TME of cGAS-expressing tumors. Second, to investigate immunological consequences of cancer-cell-intrinsic cGAS expression in advanced cancer, we generated LLC cells with inducible cGAS-expression.

In contrast to studies showing that administration of STING agonists in the TME reduces tumor progression, direct STING activation in T cells inhibits their survival, proliferation and function (Sivick K E, et al., Cell Reports 2018). Our preliminary data confirm that STING signaling reduced the proliferation of T-cells after TCR-stimulation *in vitro*. To further investigate the relevance of STING signaling in T cells for anti-tumor immunity, we will use tumor models in mice with T cell-specific STING deficiency.

Our results will lead to a better understanding of how cancer-cell-derived cGAMP influences the TME and thus anti-cancer immunity.

CanIsoNet v1.0: Database to dissect the functional impact of Isoform Switching Events in Cancer

Tülay Karakulak[1, 2, 3], Damian Szklarczyk[2, 3], Christian von Mering[2, 3], Abdullah Kahraman[1, 2, 3]

[1]Department of Pathology and Molecular Pathology, University Hospital Zurich, Zurich, Switzerland, [2]Institute of Molecular Life Sciences, University of Zurich, Zurich, Switzerland., [3]Swiss Informatics Institute, Swiss Institute of Bioinformatics, Lausanne, Switzerland

Alternative splicing is an essential regulatory mechanism in mammalian cells contributing to protein diversity. This regulation is frequently disturbed in cancer mainly due to mutations in cis-acting sequences, alterations, or expression changes in splicing regulators. Disruptions in alternative splicing regulation can cause overexpression of an alternative isoform, which can alter protein interaction network and induce cancer progression and metastases. We have recently analyzed the pathogenic impact of isoform switching events in 1209 cancer samples covering 27 different cancer types. Expanding on those results, we have built a user-friendly Cancer Isoform Specific Interaction Network (CanIsoNet) database to make our results more accessible to the cancer research community. CanIsoNet runs on a MySQL database with a Python Flask front end holding a rich set of annotations on over 120000 isoform switching events including network visualizations for 93738 isoforms using STRING. CanIsoNet provides two main functionalities to the users: 1) Browsing various statistics on cancer-specific isoform switching events in each 1209 cancer samples from the Pan-Cancer Analysis of Whole Genomes (PCAWG) project 2) Exploring and visualizing network disruptions in the STRING interaction network. We believe that CanIsoNet is an important resource for the cancer research community and will serve as a gateway to better understand the functional role of cancer-specific alternative splicing events.

The Hypoxic Response in Natural Killer Cell- Mediated Tumour Surveillance

Ekaterina Khatchatourova[1], Ewelina Krzywinska[1], Veronika Sexl[2], Christian Stockmann[1]

[1]Institute of Anatomy, University of Zurich, Winterthurerstrasse 190, Zurich CH-8057, Switzerland, [2]Institute of Pharmacology and Toxicology, University of Veterinary Medicine Vienna, Veterinärplatz 1, Vienna 1210, Austria

Natural killer (NK) cells are members of the innate immune system with potent effector function against transformed cells. The ability of NK cells to kill target cells directly without prior sensitization makes them attractive candidates for cell-based immunotherapy. However, in contrast to haematological malignancies, only a limited anti-tumour effect is observed following NK cell transfer in patients with solid tumours. This is likely due to impaired NK cell infiltration, survival and performance within the hypoxic tumour microenvironment.

Hypoxia induces the Hypoxia-Inducible Factor (HIF) pathway, and we have previously shown that NK-cell-specific deletion of HIF-1 leads to reduced NK cell counts in the spleen and to impaired NK cell activation and effector function. We now show in a gain of function approach that constitutive HIF expression improves survival of NK cells: whereas wildtype NK cells require IL-15 for long-term survival and proliferation, HIF overexpression renders NK cell survival and proliferation independent of IL-15. Forced HIF stabilization also enhances NK cell cytotoxicity, as evidenced by increased expression of cytotoxic FasL and Granzyme B (GzmB). Finally, this is associated with increased cytotoxicity against YAC-1 tumour cells *in vitro*. Thus, boosting the hypoxic response enhances NK cell expansion and effector function.

Taken together, these findings suggest the potential use of HIF-stabilizing compounds to boost NK cell performance in the adoptive transfer setting.

Impact of Ionizing Radiation on the Energy Metabolism of Healthy and Tumor Cells in the Brain

Marvin Kreuzer[1], Martin Pruschy[1], Bruno Weber[2]

[1]Department of Radiation Oncology, [2]Institute of Pharmacology and Toxicology

Metabolic reprogramming of tumor cells is considered one of the hallmarks of cancer. Studies have shown that abnormal activation of oncogenes and cancer related signaling pathways as well as the inactivation of tumor suppressor genes can induce the metabolic reprogramming in tumor cells. These metabolic changes facilitate rapid proliferation, continuous growth, survival in harsh conditions, invasion, metastasis, and immune evasion. Following the application of radiotherapy, the activity of several metabolic pathways significantly changes, potentially leading to the development of radioresistance. However, a differential effect of ionizing radiation on tumor and healthy cells is not well understood. Our goal is to collect quantitative data on the metabolic changes in healthy and tumor brain cells in response to irradiation to better understand the effect of ionizing radiation on the energy metabolism on a molecular level. In collaboration with Prof. Dr. Bruno Weber, we are developing a novel tool that will allow to measure dynamic metabolite changes in healthy and tumor brain cells upon irradiation in vivo and in real time. This tool combines imaging of recently developed fluorescent biosensors based on the Förster Resonance Energy Transfer and millimeter-scaled irradiation. Moreover, our research aims at identifying the global metabolic signature of brain tumor cells and healthy astrocytes before and after irradiation to identify pathway changes correlated with radiosensitivity. We perform global metabolomic and lipidomic analysis using liquid chromatography coupled to mass spectrometry in collaboration with the Functional Genomics Center Zurich. These methods are expected to provide knowledge to increase the efficiency of radiotherapy.

The neural crest stem cell marker NGFR confers immune evasion to melanoma cells

Julia Lehmann[1], Johanna Diener[1], Nicole Caduff[2], Ewelina Krzywińska[1], Christian Stockmann[1], Christian Münz[2], Lukas Sommer[1]

[1]Institute of Anatomy, University of Zurich, [2]Institute of Experimental Immunology, University of Zurich

Cutaneous melanoma, the deadliest type of skin cancer, is a very heterogenous and plastic tumor with a high potential to metastasize. Metastasis formation has been linked to stem cell-like subpopulations of melanoma cells, which (amongst others) express the neural crest stem cell marker NGFR/CD271/P75NTR. However, the molecular mechanisms by which NGFR drives metastasis formation remain largely unknown.

Interestingly, transcriptome analysis of NGFR overexpressing human melanoma cells revealed a putative immune evasion signature, suggesting immunosuppression as a potential mechanism for NGFR-driven metastasis formation. In line with this hypothesis, we found substantially less tumor infiltrating innate immune cells in xenograft tumors of NGFR overexpressing human melanoma cells, - with a significant decrease in NK cells. Using *in vitro* cytotoxic assays, we could demonstrate that melanoma cells with endogenously or induced high NGFR levels got less killed by allogeneic and KIR-matched NK cells than control cells with low NGFR levels. The same held true *in vivo* in that NGFR overexpressing melanoma cells got specifically less killed when injected together with human NK cells into the peritoneum of NSG mice. Finally, in an *in vivo* metastasis assay, where human NK cells were adoptively transferred into NSG mice, NGFR overexpressing melanoma cells formed more metastases in the lungs compared to control cells.

Together, these findings suggest an immune evasive role of NGFR that protects melanoma cells from immune eradication and thus facilitates metastasis formation.

MSH6 depletion promotes tumor growth in a RCAS/tv-a glioblastoma mouse model

Roxanne Lourman[1], Franz Szulzewsky[2], Eric C. Holland[2], Michael Weller[1], Hans-georg Wirsching[1]

[1]Laboratory of Molecular Neuro-Oncology, University Hospital Zurich and University of Zurich, Switzerland, [2]Human Biology Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA.

The DNA repair protein O-6-methylguanine-DNA methyltransferase (MGMT) is the most relevant prognostic marker for treatment response to temozolomide (TMZ)-based alkylating chemotherapy in glioblastoma, the most aggressive primary brain tumor in adults. Despite the availability of a standard-of-care treatment combining surgical resection followed by TMZ and radiation therapy, most glioblastoma patients succumb to treatment-resistant tumor recurrence. Recent clinical studies indicate that mismatch repair (MMR)-deficient tumors define a patient subgroup with a particularly poor prognosis. It is hypothesized that in absence of MGMT caused by promoter methylation, MMR deficiency counteracts TMZ by maintaining DNA replication despite TMZ-induced DNA lesions, leading to accumulation of somatic mutations. Using a newly developed RCAS/tv-a mouse model, we modelled aberrations in PDGF signaling, *PTEN* loss, *MGMT* promoter methylation and p53 pathway alterations, which comprise the most common genetic alterations found in glioblastoma patients. Using this unique model, we discovered that Msh6 depletion promotes tumor growth in mice independent of Mgmt expression or treatment with chemoradiation. *In vitro*, shRNA-mediated knockdown of *MSH6* resulted in increased proliferation of patient-derived glioma-initiating cells. Accordingly, *MSH6* deficiency is associated with a reduction of S-phase cells, indicating impaired post-replicative MMR, even in the absence of treatment. These results indicate a novel role of *MSH6* deficiency as a driver of tumor growth independent of its role in counteracting alkylating chemotherapy, which can be used to identify and target specific vulnerabilities of this tumor subtype.

Mechanisms of Tumor Recurrence and Drug Resistance in Rhabdomyosarcoma

Devmini Moonamale[1], Marco Wachtel[1], Beat Schäfer[1]

[1]University Children's Hospital Zurich, Balgrist Campus, Lengghalde 5, 8008 Zürich, Switzerland

Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in children, accounting for 4-8% of all paediatric cancers. The two main histological subtypes are alveolar RMS (aRMS), driven by a fusion protein PAX3-FKHR and embryonal RMS (eRMS), which is genetically heterogeneous. The prognosis for RMS has improved over the years, however the cure rates for recurrent disease remains less than 30%. In order to address this issue and find novel therapeutic targets it is necessary to identify the mechanisms underlying tumour recurrence and drug resistance in RMS.

In this project, we aim to identify cellular mechanisms that confer resistance to chemotherapy. To this end we performed two *in vitro* CRISPR screens using an sgRNA library targeting the kinome and another one targeting the whole-genome, in a patient derived xenograft (PDX) cell model that originated from a relapsed eRMS tumour. The screen was performed in combination with chemotherapy in order to identify pathways involved in promoting resistance to etoposide, which is commonly used to treat patients with relapsed eRMS. Gene lists were generated for both screens in order to choose hits with the most significance.

Therefore, to further investigate the role of the top hits, in conferring resistance to chemotherapy, we will individually validate the hits in different PDX cells as well as cell lines in combination with chemotherapeutic drugs used to treat relapsed eRMS. With this approach we hope to identify a novel combination therapy for relapse eRMS patients.

Characterization of the Writer and selected Targets of Mitochondrial ADP-ribosylation and their Role in Aging and Cancer

Lukas Muskalla[1], Michael O. Hottiger[1]

[1]Department of Molecular Mechanisms of Disease (DMMD), University of Zurich

Mitochondria are best known for their metabolic functions generating the majority of the cellular energy, earning them the name as powerhouse of the cell. While the major metabolic processes in mitochondria are well established, the regulation of these processes is currently heavily investigated. One significant way to regulate protein function is via posttranslational modifications. One of the more recently described PTMs in mitochondria is ADP- ribosylation. Our laboratory recently provided strong evidence for the existence of mitochondrial ADP-ribosylation by immunofluorescence using a novel antibody against ADP-ribose. Moreover, mitochondrial ADP-ribosylation was quantified by quantitative high-content microscopy and further confirmed by western blot, and mass spectrometry.

Based on preliminary data on the characterization of mitochondrial ADP-ribosylation, we hypothesize that mitochondrial ADP-ribosylation is catalyzed by one or more enzymes (i.e. writer(s)). Further, we propose that mitochondrial ADP-ribosylation regulates the enzymatic activity of the modified target proteins and protein complexes, thereby regulating metabolic processes. Based on this hypothesis, we propose that mitochondrial ADP-ribosylation plays an important role in the development and progression of mitochondria-associated diseases. Therefore, we will investigate these aspects also in the context of cellular aging and cancer, two pathologies that are accompanied by alterations of the mitochondrial function and structure.

The immune regulation of liver metastasis

Marc Nater[1, 2], Giulia Lucchiari[1, 2], Laura Ducimetière[1, 2], Michael Brügger[2, 3], Tomas Valenta[2, 3], Sonia Tugues[1, 2], Maries van den Broek[1, 2]

[1]Institute of Experimental Immunology, University of Zurich, Switzerland, [2]Comprehensive Cancer Center Zurich, Switzerland, [3]Department of Molecular Life Sciences, University of Zurich, Switzerland

The liver is a major metastatic target organ, and little is known about the role of immunity in controlling hepatic metastases. Compared to other organs, the liver harbors a unique immune environment characterized by the abundance of innate cell lineages including conventional natural killer (cNK) cells, tissue-resident type 1 innate lymphoid cells (trILC1s) and natural killer T (NKT) cells. Using an experimental model of liver metastasis based on the intrasplenic injection of tumor cells, we have shown that the concerted and non-redundant action of cNK cells and trILC1s is essential for anti-metastatic defense. While trILC1 controlled metastatic seeding, cNKs restrained outgrowth (Ducimetière, Lucchiari et al. (2020), bioRxiv). By contrast, NKT cells seemed to promote the formation of metastasis in the same model, presumably through the regulation of cNK and trILC1s activity. The underlying mechanisms, however, are largely unknown.

To investigate how different innate cells interact and affect metastatic progression in a more physiologically relevant setting, we have established an orthotopic model of colorectal cancer that spontaneously metastasizes to the liver within 6 weeks. Using high-dimensional flow cytometric profiling and transcriptomic analyses, we will characterize the immune microenvironment of metastatic liver from genetically modified mouse strains, which are deficient for different populations of the innate lymphoid compartment. This knowledge will improve our understanding of the immune regulation of metastasis and may lead to discovery of novel therapeutic targets.

Spermidine ameliorates colitis and induces anti-inflammatory macrophages

Anna Niechcial[1], Marlene Schwarzfischer[1], Marcin Wawrzyniak[2], Kirstin Atrott[3], Andrea Laimbacher[2], Michael Scharl[4], Marianne R. Spalinger[5]

[1]Msc, [2]Dr., [3]Ms, [4]Prof., [5]PD Dr.

Inflammatory bowel diseases, including ulcerative colitis and Crohn's disease, are chronic, inflammatory disorders of the gastrointestinal tract, with strong innate immune contribution. Due to persistent inflammation and repeated epithelial damage, IBD patients are at higher risk to develop colorectal carcinoma, one of the most frequent cancers worldwide. Thus, novel therapeutic strategies to treat chronic inflammation are an urgent need not only to improve the live quality of IBD patients, but also to prevent the development of colorectal carcinoma. We have previously demonstrated that the naturally occurring polyamine spermidine reduces severity of chemically induced colitis in mice. Here we expand on these findings and investigated the effect of spermidine in T cell mediated colitis. In an adoptive T cell transfer colitis model, spermidine treatment significantly ameliorated disease severity (reduced weight loss, decreased endoscopic colitis-scores, absence of colon shortening, lower histological inflammation-scores). While spermidine treatment only moderately affected T cell subsets, it reduced the abundance of pro-inflammatory (M1) macrophages, whereas alternatively activated (M2) macrophages were elevated in the colon of spermidine treated mice. *In vitro*, spermidine treatment of bone marrow-derived macrophages resulted in a significant reduction of *H2-ab1*, *Cd86* and *Nos2* mRNA expression, and an upregulation of *Mrc1* gene, indicating that spermidine might directly influence macrophage polarization. This demonstrates the important role of macrophages in mediating the anti-inflammatory effect of spermidine, likely via promoting anti-inflammatory macrophage development/polarization. Summarized, these findings indicate that spermidine supplementation might be a promising novel therapeutic option for the treatment of intestinal inflammation.

Nuclear Actin Polymerisation Dictates the Immediate Cellular Response to DNA Replication Stress

Maria Dilia Palumbieri[1], Thomas von Känel[1], Svenja Ulferts[2], Gabriel Senn[1], Robert Grosse[2], Massimo Lopes[1]

[1]Institute of Molecular Cancer Research, University of Zürich, [2]InInstitute of Experimental and Clinical Pharmacology and Toxicology, Albert-Ludwigs-Universität Freiburg

One of the earliest events during tumorigenesis is DNA replication stress (RS), indicating the transient stalling or pausing of replication forks facing DNA lesions, replication-transcription conflicts or other hindrances to DNA synthesis. Numerous cellular mechanisms have evolved to respond to RS, with the ultimate goal of preserving genome stability. Replication fork reversal (RFR) – i.e., the remodelling of replication forks into 4-way junctions – has emerged as a global and genetically controlled response to various forms of endogenous and exogenous RS. Importantly, our lab has shown that RFR extends throughout the nucleus, also to forks not directly challenged by DNA lesions. However, how such global regulation is accomplished in the complex 3D organisation of the nucleus is still unknown.

As nuclear actin filaments (F-actin) were recently involved in DNA repair mechanisms, we tested a possible role of F-actin in the RS response. Using specific tools for genetic or chemical inactivation, we found that nuclear F-actin is strictly required to mediate active fork slowing and remodelling upon clinically relevant genotoxic treatments. We are now attempting to visualize which F-actin structures mediate this early RS response. We are also probing direct interactions of F-actin with the replication machineries and/or a functional role of F-actin in the recruitment of specialised fork remodelling proteins to replication factories.

Our ultimate goal is to provide novel insight into global pathways coordinating RS and dynamics throughout the nucleus, thus contributing to the molecular understanding of clinically relevant conditions of RS in cancer onset or therapy.

Senataxin preserves genome integrity by counteracting R-loop accumulation

Satyajeet Rao[1], Esin Isik[1], Pavel Janscak[1]

[1]Institute of Molecular Cancer Research, University of Zürich, Switzerland

DNA replication and transcription are highly coordinated events which are spatiotemporally separated through the cell cycle. However, various endogenous events such as oncogene activation, nucleotide pool depletion, or formation of G-quadruplex structures can trigger head-on transcription-replication collisions (TRC) associated with the generation of co-transcriptional RNA:DNA hybrids termed as R-loops. R-loops act as a physical roadblock to replisome progression and have been established to be a major cause of genomic instability. Here, we report that Senataxin, a putative RNA:DNA helicase, acts as a dominant factor promoting replication fork progression by preventing R-loop accumulation. We find that Senataxin-deficient cells exhibit increased TRCs and that Senataxin is indispensable for fork restart at the site of these TRCs. As expected, Senataxin-deficient cells exhibit R-loop-dependent chromosome segregation defects. Interestingly, Senataxin-deficient cells exhibit fork-remodeling-independent nascent DNA degradation (NDD) phenotype. We show that this phenomenon is a consequence of R-loop accumulation and depends on active transcription. Our data suggest that NDD in Senataxin-deficient cells initiates from MUS81-cleaved replication forks, which accumulate due to inefficient fork restart. Our findings have implications for development of new therapeutics for treatment of Senataxin-mutated cancers and neurodegenerative diseases such as ALS4 and AOA2.

Exploring the immune response after locoregional treatment of peritoneal metastasis

Lilian Roth[1], Linda Russo[1], Chantal Pauli[2], Eva Breuer[1], Pierre-Alain Clavien[1], Anurag Gupta[1], Kuno Lehmann[1]

[1]Department of Surgery and Transplantation, University Hospital Zurich,
[2]Department of Pathology, University Hospital Zurich

The combination of cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC) has improved survival of selected patients with peritoneal metastasis (PM) from colorectal cancer. Nevertheless, peritoneal recurrence, presumably due to remnant cancer cells, is common and requires further optimization of this locoregional treatment. Therefore, it is important to understand mechanisms operating behind HIPEC. We hypothesize that HIPEC might not only be cytotoxic, but may also induce immunity. Immunogenic changes were assessed in-vitro on cancer cell-lines and patient-derived organoids. Functional consequences on the immune response were in-vivo investigated.

Cancer cell-lines and organoids were treated with hyperthermic chemotherapy for 30 minutes. Immunogenic changes (CTA - cancer -testis antigen and MHC-I expression) were assessed with flow cytometry, RT-PCR and WB. The induced PM mouse model was treated with a single application of MitomycinC/Doxorubicin intraperitoneal . Peritoneal tumor was harvested for FACS analysis and histology. Tumor load was assessed with the murine peritoneal cancer index (PCI).

Mitomycin C/Doxorubicin and Oxaliplatin in HIPEC-like condition increased the expression of up to 6 CTAs. Furthermore, MHC-I molecule expression was significantly increased after treatment. The hyperthermic chemotherapy application also increased the expression of CTAs on organoids from PM-lesions. The single application of MitomycinC/Doxorubicin resulted in a significant decrease of the PCI. Furthermore, the treatment attracted significantly more CD8+ T-cells into tumor lesions. These CD8+ T-cells were CD39 positive and less PD1 positive.

Hyperthermic chemotherapy application in-vitro induces CTA and MHC-I expression. The locoregional treatment of PM lesions in a mouse model attracts tumor reactive and less exhausted CD8+ T-cells.

Development of novel local treatments for peritoneal metastasis

Linda Russo[1], Roth Lilian[1], Gupta Anurag[1], Lehmann Kuno[1]

[1]Surgery and Transplantation Department, USZ

Selected patients with peritoneal metastasis (PM) arising from colorectal cancer show survival benefits when treated with the combination of cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC). Unfortunately, the limitation of this treatment is the recurrence of the disease, which is associated with poor prognosis and most likely the result of inefficient HIPEC treatment. Therefore, it is needed to understand mechanisms operating behind local treatment in order to improve therapy efficacy. One aim of my Project is to find novel drug combinations for peritoneal metastasis treatment in order to increase therapy outcome and provide long-term control of PM-lesions.

Novel drug combinations, such as standard HIPEC drugs with immunotherapies or DNA damage signalling inhibitors, were tested in vitro on human colorectal cancer cell lines (HT29) or in vivo on s.c. tumors (C57BL/6 mice). Of all novel drug combinations tested in vitro so far, Oxaliplatin in combination with the inhibition of the DNA damage transducer ATR, resulted in a synergistic therapeutic efficacy. This synergistic effect will further be investigated in-vivo. Oxaliplatin treated s.c. tumors showed only minimal effects on tumor growth, whereas its combination with anti PD-1 antibody caused strong tumor remission. The synergic effect was found to be associated with more activated (CD39 marker) and less exhausted intratumoral CD8 T cell (PD-1 marker). These findings suggest that increased therapy efficacy can be achieved by the combination with immunotherapy. Further experiments will explore the activation of CD8+ T-cells after single chemotherapy application, multiple application and combination with immunotherapy in a PM tumor-model.

Developing A Noninvasive Biomarker Assay For Early Detection of Colorectal Cancer

Sija Sajibu[1], Stephany Orjuela[1, 2]

[1]IMCR, [2]MLS

Colorectal cancer (CRC) is the third most commonly diagnosed cancer in the world and is one of the leading causes of cancer-related death. Colonoscopy, the standard CRC screening test, while being sensitive, is also invasive, expensive, require much preparation, and involves risk of complications. Despite the availability of many noninvasive stool tests for the patients, these tests are still not on par with colonoscopy concerning the sensitivity in detecting tumors. Thus, my overall goal is to design an accurate and noninvasive stool test based on the detection of age-independent, differentially methylated regions (DMRs) in the fecal DNA, which would detect the presence of a tumor in the colorectum and, consequently, to improve survival rates. With qPCR, our results indicate that the proportion of human DNA is only 0.2% in the total fecal DNA sample. Nanopore sequencing on the fecal sample revealed that the fragment size of the fecal DNA molecules was below 5000bp, peaking around 700bp. With the size selection, we were able to enrich for fragments below 5000bp and increase the proportion of human DNA by 3-fold. Even though, there is a further enrichment of human DNA with methyl-binding domain proteins with qPCR, the alignment to the human genome is still low and varies across the different samples. Current work aims to establish a more targeted multiplex bisulfite PCR sequencing approach to investigate 20 CRC specific DMRs in the fecal DNA.

Unravelling the mechanisms of immunomodulation in breast cancer

Tatjana Schmitz[1]

[1]University of Zürich, Department of Quantitative Biomedicine

Female breast cancer is the fifth leading cause of cancer death worldwide. A major obstacle of successful therapy is the heterogeneity in the tumor ecosystem. In breast cancer, three different tumor immune groups (TIGs) were identified in a recent study by J. Wagner et al. (2019). The TIG1 is less infiltrated by immune cells, while in TIG2 and TIG3 various leukocytes are present. TIG2 exhibit high frequencies of Tregs, PD-L1+ TAMs and PD-1highCTLA-4+CD38+ exhausted T cells indicating an immunosuppressive environment. It is suggested that dysfunctional T cells are highly tumor reactive and their capacity to target tumor cells might be reactivated by immune checkpoint blockade (ICB). To study the potential of exhausted T cells to eliminate breast cancer cells, an organoid model co-cultured with patient-matched peripheral blood mononuclear cells (PBMCs) will be used. Thereby, we aim to recapitulate the immune suppressive capacity of TIG2 tumors and the behavior of the immune cells, as well as the inter- and intra-patient heterogeneity. Suspension mass cytometry (SMC) will be applied on the mixed population, which allows us to stain for up to 40 markers at the same time and to monitor expression of immunomodulatory markers by cancer and exhaustion and activation markers by immune cells. Together with comprehensive methods like multispectral time lapse imaging to observe behavior and image-based mass cytometry (IMC) to capture the spatial distribution of markers, we aim to unravel the mechanisms of immunoediting in breast cancer.

The molecular landscape of proteasome inhibitor resistance in multiple myeloma

Jonas Schwestermann[1]

[1]Kantonsspital St. Gallen

Multiple Myeloma (MM) is a hematological malignancy characterized by clonal proliferation of malignant plasma cells within the bone marrow. MM remains an incurable malignancy, with most patients relapsing and dying from the disease. Anti-myeloma drugs such as proteasome inhibitors (PIs) have considerably improved prognosis in myeloma. Despite these advances, disease heterogeneity, early relapse and treatment resistance still pose major challenges in MM treatment. To study the molecular dynamics of PI-resistant MM *in vivo,* we established a mouse model, where RPMI-8226 cells were injected intrafemorally and tumor progression was monitored using a non-invasive imaging system. Several mice were treated with the PI Carfilzomib (CFZ), whereas others remained untreated. After long-term exposure to CFZ, RPMI-8226 cells eventually became drug resistant. At this point, RPMI-8226 cells were isolated and processed for single-cell RNA sequencing with the aim to characterize a transcriptional CFZ-resistance signature in refractory cells, when compared to untreated cells. To investigate the role of tumor microenvironment (TME)-associated human stromal cells (HS5) towards CFZ resistance, we performed a genome-wide CRISPR/Cas9 library screening where Brunello library transduced RPMI-8226 cells were co-cultured with HS5 cells and exposed to increasing concentrations of CFZ to identify sensitivity and resistance candidate genes. Lastly, we performed a subsequent CRISPR/Cas9 library screening, where Brunello library and synthetic Notch receptor (synNotch) transduced HS5 cells were co-cultured with synNotch ligand transduced RPMI-8226 cells to identify genes that are essential for cell-cell interactions. Together, this study aims to identify new molecular pathways and develop innovative treatment strategies to overcome PI-resistant myeloma.

Dual role of tumor cell-derived IL-10 in promoting cell-autonomous growth and immune escape in diffuse large B-cell lymphoma

Kristin Stirm[1]

[1]Institute of Molecular Cancer Research

Diffuse large B-cell lymphoma (DLBCL) is an aggressive malignancy arising from germinal center or post-germinal center B-cells that retain many of the properties of normal B-cells, including their reliance on growth-promoting cytokines and other cues provided by the tumor microenvironment. Here we show that a subset of DLBCL express the cytokine IL-10 and its receptor, which activates STAT3 phosphorylation and expression of STAT3 target genes. Genetic deletion of the unique signaling chain of the IL-10R, IL-10RA, abrogates the autocrine STAT3 phosphorylation triggered by tumor cell- intrinsic IL-10 expression and impairs growth of DLBCL cell lines in subcutaneous and orthotopic models. Furthermore, we demonstrate here using a syngeneic, MYC-driven model of DLBCL that antibody-mediated neutralization of IL-10 signaling reduces tumor growth, which can be attributed to reduced Treg infiltration, stronger intratumoral effector T-cell responses, and restored tumor-specific MHCII expression. The effects of IL-10R neutralization on MHCII expression and tumor growth were phenocopied by -but did not synergize with- Treg depletion, suggesting that tumor-derived IL-10 promotes Treg recruitment to the lymphoma microenvironment. Tumors from patients with high expression of IL-10RA are infiltrated by higher numbers of Tregs than IL-10RAlow patients. Finally, we show in 16 cases of DLBCL derived from patients on immunosuppressive therapy after solid organ transplantation that IL-10RA expression is less common in this cohort, and Treg infiltration is not observed. In conclusion, we report here a dual role for IL-10 in cell-autonomous growth of DLBCL cells on the one hand, and immune evasion through Treg recruitment on the other.

Endogenous Retrovirus expression activates type-I interferon signaling in an experimental mouse model of mesothelioma development

Suna Sun[1], Francesca Frontini[1], Weihong Qi[2], Ananya Hariharan[1], Manuel Ronner[1], Martin Wipplinger[1], Christophe Blanquart[3], Hubert Rehrauer[2], Jean-François Fonteneau[3], Emanuela Felley-Bosco[1]

[1]Laboratory of Molecular Oncology, Department of Thoracic Surgery, Lungen- und Thoraxonkologie Zentrum, University Hospital Zurich, [2]Functional Genomics Center Zürich, ETH Zürich/University of Zürich, Zürich, Switzerland, [3]Université de Nantes, CNRS, INSERM, CRCINA, F-44000 Nantes, France

Early events in an experimental model of mesothelioma development include increased levels of editing in double-stranded RNA (dsRNA). We hypothesised that expression of endogenous retroviruses (ERV) contributes to dsRNA formation and type-I interferon (IFN) signaling. ERV and interferon stimulated genes (ISGs) expression were significantly higher in tumor compared to non-tumor samples. 12 tumor specific ERV ("MesoERV1-12") were identified and verified by qPCR in mouse tissues. "MesoERV1-12" expression was lower in mouse embryonic fibroblasts (MEF) compared to mesothelioma cells. "MesoERV1-12" levels were significantly increased by demethylating agent 5-Aza-2'-deoxycytidine treatment and were accompanied by increased levels of dsRNA and ISGs. Basal ISGs expression was higher in mesothelioma cells compared to MEF and was significantly decreased by JAK inhibitor Ruxolitinib, by blocking Ifnar1 and by silencing Mavs. "MesoERV7" promoter was demethylated in asbestos-exposed compared to sham mice tissue as well as in mesothelioma cells and MEF upon 5-Aza-CdR treatment.

These observations uncover novel aspects of asbestos-induced mesothelioma whereby ERV expression increases due to promoter demethylation and is paralleled by increased levels of dsRNA and activation of type-I IFN signaling. These features are important for early diagnosis and therapy.

Radiotherapy treatment volume in a mouse model of radiation-induced lymphopenia

Irma Telarovic[1], Jan Unkelbach[2], Martin Pruschy[1]

[1]Laboratory for Applied Radiobiology, Department of Radiation Oncology, USZ,
[2]Department of Radiation Oncology, USZ

Radiotherapy has long been classified as immunosuppressive. Recent preclinical studies have revealed that increased doses of irradiation induce potent anti-tumor immune responses, thus boosting an immense level of research at the interface of radiotherapy and immunotherapy. However, we still have limited insights into fundamental questions, such as how radiotherapy treatment volume (RTV) affects the anti-tumor immune response. As a first step towards answering this question, here we investigate the impact of RTV on the immune system of a healthy mouse.

Our mouse model of radiation-induced lymphopenia comprises five treatment groups with precisely defined RTVs, using a state-of-the art small animal image-guided radiotherapy platform and SmART-ATP treatment planning software. Based on the early timepoints, we focused our attention to the two RTVs of most interest: (1) single-field irradiation modeling a tumor irradiation with minimal extension into the normal tissue and (2) five-field irradiation extending into the abdomen and the draining lymph nodes, modeling a conservative tumor irradiation with elective lymph node irradiation. For these, we explored later timepoints and performed a detailed analysis of immune cell subtypes in lymphoid organs.

Overall, we demonstrate a strong volume-effect of irradiation on the immune system. Interestingly, even a small RTV induced significant changes. However, while the small RTV resulted in a quick recovery of leukocyte numbers, large RTV induced changes that persisted during the 21 days of follow up. Taken together, our study represents a first step towards elucidating the importance of the often-overlooked effect of normal tissue co-irradiation in the context of radio(immuno)therapy.

ADAM17-dependent paracrine and intercellular communication in response to irradiation

Fabienne Tschanz[1]

[1]University of Zurich

The cellular response to ionizing radiation (IR) depends on tumor cell intrinsic and microenvironmental factors resulting in differential radiosensitivities among different cell types. Based on an IR-induced secretome analysis we demonstrated that the activity of the matrix metalloproteinase ADAM17 is upregulated following IR in a time and dose-dependent way, correlating with a more aggressiveness. Substrates are released from the cell surface serving e.g., as receptor ligands in an auto- and paracrine way. We investigated the role of ADAM17 for the intercellular communication between tumor-cells and cells of the tumor-microenvironment in response to IR alone and in combination with ADAM17-inhibition.IR-induced migration of endothelial cells was abrogated through inhibition of ADAM17 in the attracting tumor-cells. VEGF was identified as the major factor responsible for tumor cell-directed, IR-induced endothelial cell migration and was cleaved from tumor-cells in an ADAM17-dependent way. Tumor vasculature-related in vivo endpoints showed that microvessel size and density was strongly decreased in response to the combined treatment modality of IR and ADAM17-inhibition but not by either treatment modality alone. The combination treatment resulted in significant tumor growth reduction in the subcutaneous tumor model and long-term tumor growth control in the orthotopic tumor model, monitored by sequential bioluminescence-measurements.Our data demonstrate that IR-induced, tumor-cell-associated ADAM17-activity releases VEGF and thereby coordinates the communication between the tumor-cell-compartment and the tumor-vasculature. The in vivo efficacy-oriented data suggest that the potent supra-additive effect of the novel combined treatment modality is in part due to abrogation of the ADAM17-mediated, IR-induced protective effect on the tumor-vasculature.

Pre-clinical validation and understanding the biology of spatiotemporal fractionation

Irene Vetrugno[1], Martin Pruschy[1], Jan Unkelbach[1], Nathan Torelli[1]

[1]University of Zurich

Radiotherapy is one of the central anti-cancer treatment modalities, prescribed with a curative or palliative purpose to more than 60% of cancer patients. The principal aim of radiotherapy is to induce DNA damage in the tumor, while sparing non tumorous healthy tissues from damage. Current practice minimizes the damage of organs and tissues at risk by splitting the total treatment dose into multiple fractions. The rationale of dose fractionation is justified by the improved capability of the co-irradiated healthy tissue to repair DNA damage in between treatment sessions, which leads to an increased tolerance of radiation and to an improved therapeutic window. Even though the molecular mechanisms that ensure repair of radiation damage during fractionated radiotherapy are reduced in the tumor, surviving tumor cells could still drive tumor repopulation thereby limiting tumor control. Hence, classic low dose fractionated radiotherapy regimens are primarily advantageous for the normal tissue but should be adjusted at the tumor site. We will investigate an innovative concept of fractionated radiation therapy called spatiotemporal fractionation. According to this approach, different dose distributions can be delivered in different fractions to achieve hypofractionation in the tumor while ensuring near-uniform fractionation in normal tissues. By using an image-guided high precision small animal radiotherapy platform, we will assess the projected benefit of spatiotemporal fractionation in experimental mice tumor models while unraveling the biological role of the immune system in the context of spatiotemporal fractionation.

Integrins as targets for CAR T cell therapy for glioblastoma

Danielle Villars[1], Manuela Silginer[1], Tobias Weiss[1], Michael Weller[1], Patrick Roth[1]

[1]Klinik für Neurologie

Novel therapeutic strategies for the treatment of glioblastoma are urgently needed, owing to the limited success of the current standard of care. Chimeric antigen receptor (CAR) T cell-based therapies have shown unprecedented success in haematological malignancies. Using CAR T cell therapy for the treatment of glioblastoma may be a feasible strategy to improve treatment outcomes.

Alpha v integrins are involved in various processes of tumorigenesis and are overexpressed in many types of cancer, including glioblastoma. Their expression correlates with tumor grade and overall survival and subsequently, these molecules have been regarded as promising targets for cancer therapy. Given the manageable toxicities of previously tested pharmacological integrin inhibitors, αv integrins represent targetable molecules.

Therefore, we have designed a 2nd generation CAR targeting αv-integrins, consisting of the αv-integrin scFv recognition domain, a co-stimulatory domain and a co-activating domain. The lytic activity of CAR T cells against various glioma cell lines with different antigen expression levels was tested at different effector-target ratios and analysed by flow cytometry. The αv-integrin-CAR T cells specifically lyse glioma cells in a dose-dependent and antigen-expression-level dependent manner. Additionally, we could show that the αv-integrin-CAR T cells are only activated upon antigen-recognition. In a clinically relevant, orthotopic glioma mouse model, we could also confirm the anti-glioma activity of the CAR T cells *in vivo*.

The xenograft model limits the study of treatment-related toxicities, thus we are trying to generate an equivalent CAR T cell system in a syngeneic mouse model.

Quantification of Protein Degradation Rate Constants in the Living Cell

Dorothea Winkelvoss[1]

[1]UZH, Plückthungroup, same as David Vukovic

Today's therapies are mostly based on the binding of a drug to target proteins. For clinically relevant effects to manifest, drugs need to bind a significant portion of target protein for extended periods of time. A novel, event-based approach is represented by a class of drugs that, upon binding, result in the enzymatic destruction of their target protein. Such drugs can engage in multiple rounds of target destruction, alleviating the limiting paradigm of a 1:1 binding ratio and result in biological effects at lower site of action concentrations.

Our aim is to engineer bispecific molecules enabling the destruction of a chosen intracellular protein through redirecting the cell's own protein degradation machinery. Target specificity should be readily exchanged using antibody-like protein scaffolds. Engagement with the ubiquitin-proteasome system (UPS) is achieved by adding E3-ligase domains, degrons, or small-molecules via genetic fusion or chemical coupling, respectively.

In order to truly engineer such molecules, it is necessary to experimentally dissect a multitude of parameters to quantify degradation-rate constants. Important aspects include autoubiquitination, degradation-properties of binder engagement, binding affinity, protein stability as well as fair comparisons of UPS-interacting approaches. Our experimental approach consists of a combination of adherent cell microinjection, simultaneous epifluorescence and confocal fluorescence microscopy in the living cell. The resulting large amounts of data are processed with a custom data analysis pipeline involving R statistical environment, CellProfiler and Matlab.

Our approach elucidated unknown degradation-inducing properties of eGFP-binding proteins and confirmed catalytic behavior of a multitude of degradation-inducing bispecific molecules in the living cell.

Modulation of Intracellular Protein Degradation

Dorothea Winkelvoss[1]

[1]PhD student of biochemistry

The UPS system has become a platform for drug targeting. A novel therapeutic approach uses the UPS system for the enzymatic destruction of malfunctioning or over-expressed proteins by using bispecific small molecules called proteolysis targeting chimeras (PROTACs). We want to adopt the PROTAC's working principle to create a bispecific molecule using an antibody-like selected protein binder that is responsible for target binding and a small molecule, peptide or protein domain, capable of recruiting components of the cell's UPS. The use of antibody-like entities for target specificity represents a stark contrast to other approaches relying on small molecules that are currently being considered elsewhere, as protein-based approaches are independent of the target presenting a binding site for small molecules (i.e., being “druggable”). In our approach, redirecting the system against different targets is possible through a facile exchange of the target binding protein, giving the system a *completely generic character*.

New Methodology for Antibody Discovery and Engineering

Julian Weischedel[1], Laurence Higgins[1], Ufuk Karakus[2], Onur Boyman[2], Richard Chahwan[1]

[1]Institute of Experimental Immunology, UZH, [2]Department of Clinical Immunology, USZ

Antibody-based immunotherapies are becoming an effective drug modality and main pillar in cancer and inflammatory disease treatment. They can I) block essential surface receptors, II) inhibit the immunosuppressive tumour microenvironment and III) recruit and activate immune effector cells.

Currently, hybridoma and phage display technology remain standard methods for therapeutic monoclonal antibodies (mAbs) generation. Each having their own strengths and limitations. Hybridomas lead to high affinity mAbs through somatic hypermutation in animals. Hence, the process is slow and for therapeutic use these mAbs require to be humanized whereby their specificity can be lost. Phage display does not require animal models, but libraries encoding fully human antibody fragments that are screened in vitro. Yet, many antibody binders are not suitable for further applications as phage display lacks an intrinsic antibody maturation step.

Our aim is to develop a novel methodology for mAb discovery and engineering by using our own developed RCL Base Editor System. Cytosine Base Editors are a new class of gene editing tools that allow mutations at a single base resolution. In a first step we want to apply our RCL Base Editor for ex vivo mAb affinity engineering. Therefore, we started a collaboration with Prof Onur Boyman from the University Hospital Zurich. Eventually, we want to use our system to generate de novo human mAbs against cancer-related antigens.

RNA Binding Motif Protein 8A: a novel RNA editing target in mesothelioma

Martin Wipplinger[1], Asra Abukar[1], Ananya Hariharan[1], Marika Sculco[1], Manuel Ronner[1], Emanuela Felley-Bosco[1]

[1]Laboratory of Molecular Oncology, Department of Thoracic Surgery, University Hospital Zurich, Zurich, Switzerland

Malignant Pleural Mesothelioma (MPM) is a rare, aggressive cancer caused by asbestos exposure. In a mouse model of mesothelioma development, exposure to asbestos increased A-to-I RNA editing paralleled by an increase in RNA editing enzymes, adenosine deaminases acting on RNA (ADAR1, ADAR2) and high ADAR2 mRNA levels are associated with worst overall survival in MPM patients. We found that RNA binding motif protein 8a (RBM8a) is edited upon mesothelioma development and functions as an essential gene in a BRCA-associated protein 1, a prominent tumour suppressor gene in MPM, proficient mesothelioma model. RBM8a is part of the Exon Junction Complex, which mediates splicing, transport and translation of mRNA and controls the nonse-mediated RNA decay. Further, it was shown to be involved in treatment resistance. In our study, we aim to better characterize RBM8A in MPM and the effect of RNA editing on RBM8a expression.

In MPM cells we observed significantly increased RBM8a protein levels as well as A-to-I editing levels within repetitive Alu elements of the 3'UTR compared to normal mesothelial cells. ADAR2 overexpression in normal mesothelial cells increased RBM8a editing and protein levels. In a luciferase-reporter system unedited, but not edited Alu elements, were more stable in mesothelioma when compared to normal mesothelial cells.

We conclude that RNA editing increases RBM8a protein expression. This is possibly mediated by the binding of an RNA binding proteins (RBP) to the edited 3'UTR in MPM.

Extracellular vesicles influence B cell maturation signaling

Kevin Yim[1]

[1]Institute of Experimental Immunology

B cell maturation is an essential process for effective adaptive immunity. This process requires a complex signaling network to precisely control critical genes' expression and mediate the mutagenic process necessary for antibody diversification. B cells also require extracellular signaling queues from other cells within the germinal center. Recently, a novel class of intercellular signaling mediated by extracellular vesicles (EVs) has emerged. Studies have shown B cell EVs mediated signaling is involved in immune response regulation, infection control, and tumorigenesis. However, the role of B cell EVs in B cell maturation is not yet established. We herein study B cell EVs' biological properties and physiological function in the context of B cell maturation. We use novel technologies to characterize B cell EVs surface marker signatures, molecular cargo and physiological roles in B cell maturation. A new specialized nanoparticle analyzer was used to profile B cell EVs at the single-particle level and characterize their surface markers and sub-populations at various maturation stages. EV ncRNA cargo was characterized by RNA-seq and bioinformatic analyses identified an EVs mediated regulatory network for B cell maturation. The physiological role of EVs in B cell maturation is investigated using EV blockade assays and complementation studies using diverse EV sources further confirmed the physiological role and mode of action of EVs in B cell maturation.

Mass Spectrometry-empowered Spatial Single-Cell Proteomics Techniques in Cancer Research

Mengze Zhang[1]

[1]University of Zürich, Department of Quantitative Biomedicine

Revealing the spatial distribution of components within cancerous tissues at the single-cell level is of the core interest of many cancer researchers. Thanks to the rapid development of single-cell technology and the enriching toolbox of imaging techniques, such needs are largely fulfilled. Nonetheless, the technical progress is disproportionally attributed to the genomics field; while the analytical capacity of post-genomics, especially proteomics tools, are lagging behind. Nowadays, the most often-applied techniques for single-cell proteomics are immunofluorescence microscopy (IF) and Imaging Mass Cytometry (IMC). Although the later one transformed imaging multiplexity to ~40 parameters detection, such performance is insufficient for a comprehensive depiction.

Mass Spectrometry (MS) profiles thousands of proteins simultaneously, hence being seen as a promising alternative to revolutionize the analysis performance. Despite it has long been recognized as a bulk analysis tool, advanced methods in Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) can now exploit over 3,000 protein identifications from a single cell. Such sensitivity enables proteome profiling of pathology–guided microdissection materials, delivering highly multiplexed readouts from a μm2-sized, relatively homogenous tissue area. Another branch of MS – Imaging Mass Spectrometry (IMS), has achieved hundreds to thousands of protein identifications from a single tissue section. With the expeditious development in methodology as well as instrumental constructions, many IMS techniques, especially Matrix-Assisted Laser Desorption Ionization (MALDI)-Imaging can now perform at the spatial resolution of less than 1 μm. Highly sensitive MS techniques are now available for facilitating cancer research, and here, discussion of the vast potentiality of their applications is highly appreciated.

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Participants

Ala'a Al Hrout
Institute of Experimental Immunology
University of Zurich
alhrout@immunology.uzh.ch

Claire Beckers
Institute of Physiology
University of Zurich
claire.beckers@uzh.ch

Erin Beebe
Institute of Veterinary Pharmacology and Toxicology
University of Zurich
erin.beebe@vetpharm.uzh.ch

Katrin Bertram
Institute of Molecular Cancer Research
University of Zurich
bertram@imcr.uzh.ch

Flurina Böhi
Department of Molecular Mechanisms of Disease
University of Zurich
flurina.boehi@dmmd.uzh.ch

Michael Brügger
Department of Molecular Life Sciences
University of Zurich
michael.bruegger2@uzh.ch

Marcel Bühler
Department of Neurology
University Hospital Zurich
marcel.buehler@usz.ch

Anna Laura Calvanese
Institute of Experimental Immunology
University of Zurich
calvanese@immunology.uzh.ch

Héctor Castañón
Institute of Experimental Immunology
University of Zürich
castanoncuadrado@immunology.uzh.ch

Karla Cervantes
Institute of Experimental Immunology
University of Zurich
cervantes@immunology.uzh.ch

Yanjiang Chen
University Hospital Zurich
University Hospital Zurich
yanjiang.chen@uzh.ch

Jing Chen
Institute of Anatomy
University of Zurich
jing.chen3@uzh.ch

Sara Danielli
Pediatric Oncology
University Children's Hospital Zürich
sara.danielli@kispi.uzh.ch

Lisa Dietsche
Department of Medical Oncology and Hematology
University Hospital Zurich
lisa.dietsche@usz.ch

Keri Fishwick
Institute of Molecular Cancer Research
University of Zurich
fishwick@imcr.uzh.ch

Anna Gramalla-Schmitz
Institute for Experimental Immunology
University of Zurich
gramalla-schmitz@immunology.uzh.ch

Corina Gsell
Institute of Veterinary Pharmacology and Toxicology
Universität Zürich
corina.gsell@vetpharm.uzh.ch

Alessandra Gurtner
Institute of Experimental Immunology
University of Zurich
gurtner@immunology.uzh.ch

Anne-Franziska Guthörl
Department of Molecular Life Sciences
University of Zurich
anne.guthoerl@uzh.ch

Ananya Hariharan
Department of Thoracic Surgery
University Hospital Zurich
ananya.hariharan@usz.ch

Mi He
Roche Innovation Center
Roche Pharma Research
mi.he@roche.com

Laura Heeb
Department of Viszeral Surgery and Transplantation
University Hospital Zurich
laura.heeb@usz.ch

Michael Herbst
Institute for Experimental Immunology
University of Zurich
herbst@immunology.uzh.ch

Mara Hofstetter
Department of Medical Oncology and Hematology
University Hospital Zurich
mara.hofstetter@usz.ch

Tulay Karakulak
Institute of Pathology and Molecular Pathology
University Hospital Zurich
tuelay.karakulak@uzh.ch

Reihaneh Khaleghi
Institute of Veterinary Pharmacology and Toxicology
University of Zurich
Reihaneh.khaleghi@uzh.ch

Ekaterina Khatchatourova
Institute of Anatomy
University of Zurich
ekaterina.khatchatourova@uzh.ch

Jenny Kienzler
Institute of Experimental Immunology
University of Zurich
kienzler@immunology.uzh.ch

Levi Kopp
Pediatric Oncology
University Children's Hospital Zürich
leviluca.kopp@kispi.uzh.ch

Marvin Kreuzer
Department of Radiation Oncology
University Hospital Zürich
marvin.kreuzer@uzh.ch

Harini Lakshminarayanan
Department of Pathology and Molecular Pathology
University Hospital Zurich
harini.lakshminarayanan@usz.ch

Julia Lehmann
Institute of Anatomy
University of Zurich
julia.lehmann@anatomy.uzh.ch

Thomas Look
Department of Neurology
University Hospital Zurich
thomas.look@usz.ch

Roxanne Lourman
Department of Neurology
University Hospital Zurich
roxanne.lourman@usz.ch

Jessica Migliavacca
Pediatric Oncology
University Children's Hospital Zürich
jessica.migliavacca@kispi.uzh.ch

Devmini Moonamale
Pediatric Oncology
University Children's Hospital Zürich
devmini.moonamale@kispi.uzh.ch

Giulia Moro
Institute of Molecular Life Sciences
University of Zurich
giulia.moro2@uzh.ch

Vaishnavi Natarajan
Institute of Molecular Cancer Research
University of Zurich
mohana@imcr.uzh.ch

Marc Nater
Institute of Experimental Immunology
University of Zurich
nater@immunology.uzh.ch

Anna Niechcial
Department of Gastroenterology and Hepatology
University Hospital Zurich
anna.niechcial@usz.ch

Maria Dilia Palumbieri
Institute of Molecular Cancer Research
University of Zurich
palumbieri@imcr.uzh.ch

Rocco Roberto Penna
Institute of Veterinary Pharmacology and Toxicology
University of Zurich
rocco.penna@vetpharm.uzh.ch

Satyajeet Rao
Institute of Molecular Cancer Research
University of Zurich
rao@imcr.uzh.ch

Lilian Roth
Department of Viszeral Surgery and Transplantation
University Hospital Zurich
lilian.roth@usz.ch

Linda Russo
Department of Viszeral Surgery and Transplantation
University Hospital Zurich
linda.russo@usz.ch

Sija Sajibu
Institute of Molecular Cancer Research
University of Zurich
sajibu@imcr.uzh.ch

Tatjana Schmitz
Department of Quantitative Biomedicine
University of Zurich/ETH Zurich
tatjana.schmitz@uzh.ch

Jonas Schwestermann
Laboratory for Experimental Oncology
Kantonsspital St. Gallen
j.schwestermann@gmail.com

Kristin Stirm
Institute of Molecular Cancer Research
University of Zurich
stirm@imcr.uzh.ch

Suna Sun
Department of Thoracic Surgery
University Hospital Zurich
Suna.Sun@usz.ch

Miaomiao Sun
Department of Neurology
University Hospital Zurich
Miaomiao.Sun@usz.ch

Irma Telarovic
Department of Radiation Oncology
University Hospital Zurich
irma.telarovic@uzh.ch

Fabienne Tschanz
Department of Radiation Oncology
University Hospital Zurich
fabienne.tschanz@uzh.ch

Irene Vetrugno
Department of Radiation Oncology
University Hospital Zurich
irene.vetrugno@uzh.ch

Danielle Villars
Departement of Neurology
University Hospital Zurich
danielle.villars@usz.ch

David Vukovic
Department of Biochemistry
University of Zurich
david.vukovic@uzh.ch

Verena Waller
Department of Radiation Oncology
University Hospital Zurich
verena.waller@uzh.ch

Remi Weber
Departement of Neurology
University Hospital Zurich
remi.weber@usz.ch

Julian Weischedel
Institute of Experimental Immunology
University of Zurich
weischedel@immunology.uzh.ch

Dorothea Winkelvoss
Department of Biochemistry
University of Zurich
dorothea.winkelvoss@uzh.ch

Martin Wipplinger
University Hospital Zürich
Laboratory of Molecular Oncology
Martin.Wipplinger@usz.ch

Cheuk Ting Wu
Department of Gastroenterology and Hepatology
University Hospital Zurich
cheukting.wu@uzh.ch

Kevin Yim
Institute of Experimental Immunology
University of Zurich
yim@immunology.uzh.ch

Milica Zecevic
Pediatric Oncology
University Children's Hospital Zürich
milica.zecevic@kispi.uzh.ch

Mengze Zhang
Department of Quantitative Biomedicine
University of Zurich/ETH Zurich
mengze.zhang@uzh.ch


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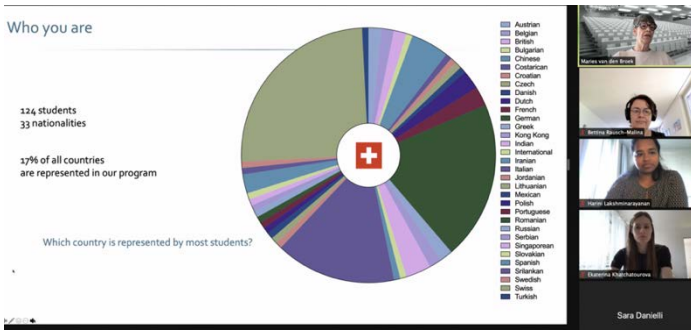
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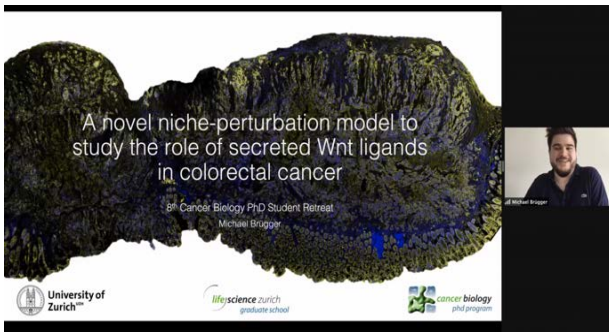
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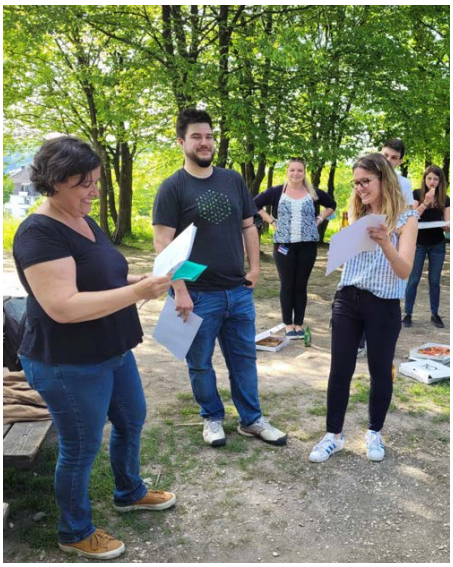


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