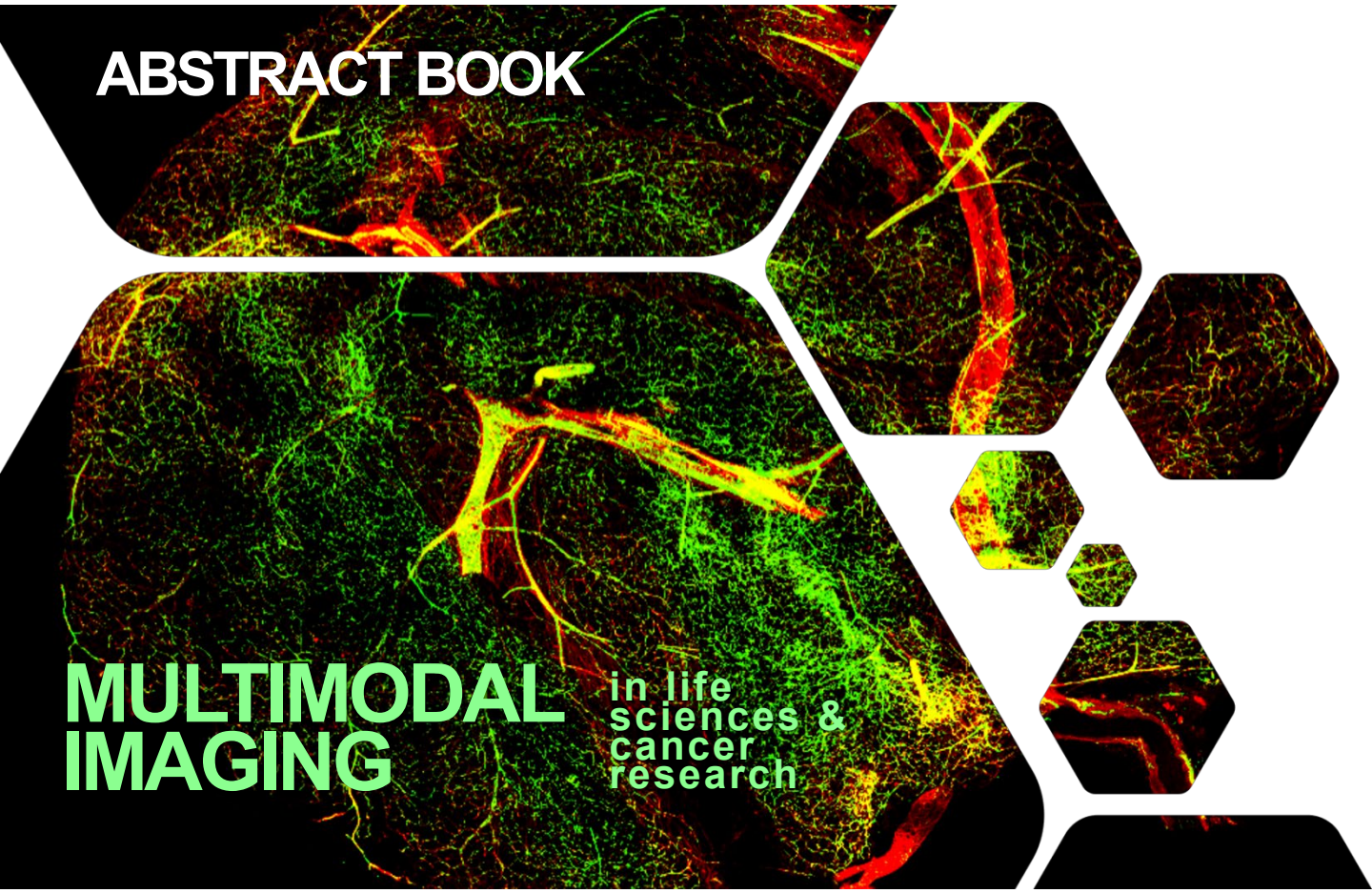

Thursday 2nd of February 2023

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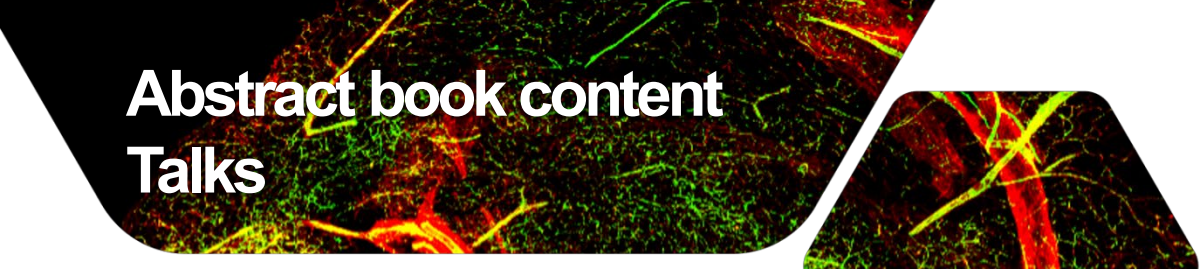

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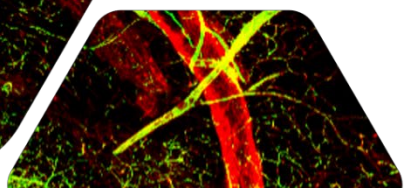

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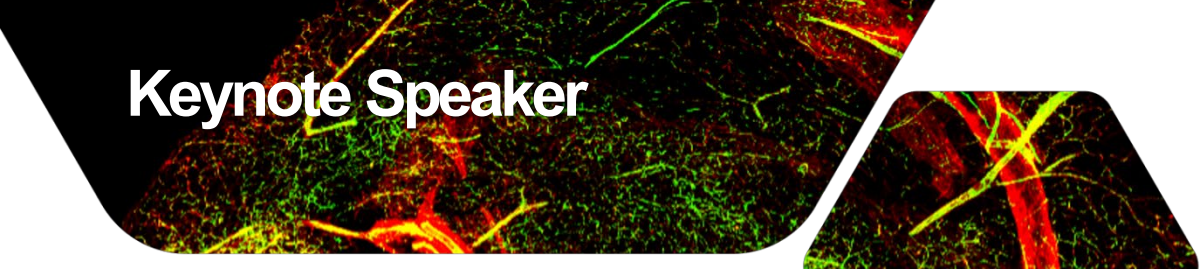


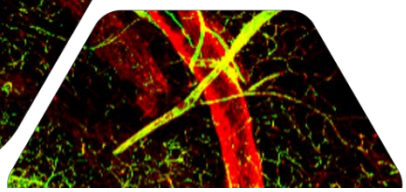
Keynote Speaker

Ralph Weissleder, Harvard/MGH USA

Imaging live: more, faster and possibly in patients

The ability to observe cells in live organisms is essential for understanding their function in complex *in vivo* milieus. A major challenge today has been the limited ability to perform higher multiplexing beyond the 4-6 “colors” to define cell subtypes *in vivo*. I will present new strategies for higher multiplexed in vitro and *in vivo* imaging than is currently possible. Based on advances in bioorthogonal chemistry, we have recently achieved 12 color in vivo imaging enabling visualization of entire immune cell repertoires, broad cell profiling in heterogenous models and imaging of drug delivery and cellular effects (PK/PD) in live mice. I will also discuss newer generations of myeloid cell targeted Therapeutics and their testing in mouse models of cancer using intravital imaging.

Session 1: Advanced Intravital microscopy

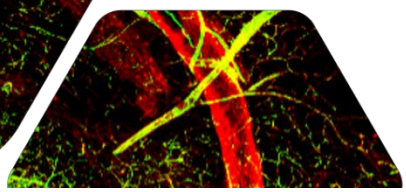


Jan Böttcher, Technical University of Munich, Germany.

Spatial and functional coordination of anti-cancer immunity by conventional type 1 dendritic cells

Initiation of anti-cancer immunity is thought to depend on priming of tumor-specific $CD8^+$ T cells by type 1 conventional dendritic cells (cDC1), but whether effective anti-cancer immunity requires cDC1 to orchestrate T cell responses within tumors remains poorly understood. Here, we use imaging-based deep learning to identify intratumoral cDC1-$CD8^+$ T cell clustering as a critical determinant of protective anti-cancer immunity in mouse cancer models. These clusters form selectively in stromal tumor regions in mouse and human cancers and constitute niches in which cDC1 amplify anti-cancer $CD8^+$ T cell responses. We identify a distinct population of tumor-resident $MHCII^{hi}CCR7^{neg}$ cDC1 that exclusively stimulate tumor-specific $CD8^+$ T cells within these niches. Mechanistically, $MHCII^{hi}CCR7^{neg}$ cDC1 produce CXCL9 to promote cluster formation and (cross-)present tumor antigens to activate and expand tumor-infiltrating $CD8^+$ T cells, which is required for cancer immune control. Our findings reveal an intratumoral phase of anti-cancer T cell responses orchestrated by $MHCII^{hi}CCR7^{neg}$ cDC1 that determines protective versus ineffective anti-cancer immunity and could be exploited for cancer therapy.

Session 1: Advanced Intravital microscopy

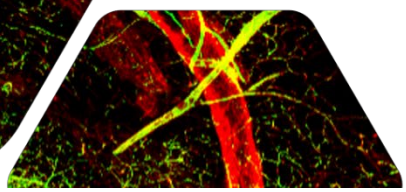


Matteo Iannacone, San Raffaele Scientific Institute Milan, Italy

Immune surveillance of the liver

I will discuss our current work trying to understand the generation of dysfunctional adaptive immune cells in chronic hepatitis B virus (HBV) infection and to develop new strategies to reprogram them into functional cells endowed with potent antiviral activity. Taking advantage of unique mouse models of HBV pathogenesis, several of which were created ad hoc, and well-characterized cohorts of patients, we dissect and target dysregulated pathways that characterize adaptive immune cell dysfunction during chronic hepatitis B. State-of-the-art static and dynamic imaging are used to analyze the behavior of adaptive immune cells ultimately differentiating into dysfunctional cells in the liver at an unprecedented level of spatial and temporal resolution. In parallel, high-dimensional flow cytometry and single-cell sequencing performed in mouse models of HBV pathogenesis and in chronically infected patients reveal the proteogenomic landscape and heterogeneity underlying adaptive immune cell dysfunction. Finally, immune-regulatory mechanisms that have been already identified or that will emerge are targeted both in vitro and in vivo. In addition to fostering new concepts in adaptive immunity and viral pathogenesis, our research has the potential to instruct the design of novel, rational strategies that direct the immune system to terminate chronic HBV infection and its attendant costs and complications.

Session 1: Advanced Intravital microscopy

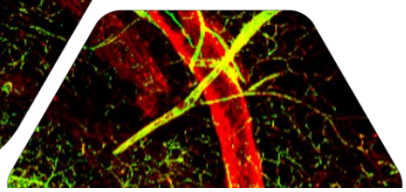


Colinda LGJ Scheele, VIB-KU Leuven Center for Cancer Biology, Belgium.

Multi-dimensional imaging of tumor initiation in the breast: how does healthy tissue structure prevent tumor formation?

Cells that have acquired mutations in driver genes are abundant in tissues of healthy individuals. In breast tissue it has been suggested that large fields of the epithelial tree can carry mutations leading to a “sick lobe”. These mutant fields may predispose to tumor formation, but in most cases mutant branches stay morphologically untransformed. The underlying mechanisms that on the one hand allow these mutant cells to stay under the radar, and on the other hand prevent cancerous cells from transformation are largely unknown. In clinical samples of non-invasive breast cancer, we combined high-resolution 3D tissue reconstruction with copy number variation sequencing and confirmed the presence of cells with oncogenic mutations in morphologically normal breast ducts, indicating that field cancerization may precede tumor formation. Using lineage tracing of mutant cells combined with intravital and whole-tissue imaging in murine mammary glands allowed us to map the dynamics by which mutant fields arise. Strikingly, in most cases, these cancerous fields did not transform the tissue. By combining quantitative modelling with 3D imaging over time, we found three mechanisms that protect the mammary epithelium from transformation: 1. **Tissue hierarchy** confers the first protection mechanism against field cancerization as only clones initiated in the stem cell compartment survive over the short-term. 2. At longer times, local tissue remodeling during the **estrous cycle** leads to stochastic collective stem cell amplification and loss. This process provides a second mechanism of protection, leading to the elimination of the majority of mutant clones, while massively accelerating the expansion of a minority of clones that, by chance, survive. 3. Eventually, this process of clone expansion becomes restrained by the one-dimensional **geometry of the ducts**, providing a third mechanism to protect the epithelium against uncontrolled colonization by mutant clones. Together, these findings reveal layers of protection that serve to eliminate the majority of cells that acquire chance somatic mutations at the expense of driving the accelerated expansion of a minority of cells, which can colonize large areas leading to field cancerization.

Session 2: Multimodal integration



Johanna Joyce, University of Lausanne, Switzerland

Dynamic and Multimodal Imaging of the Brain Tumor Microenvironment

Brain malignancies, including high-grade gliomas, are characterized by a complex tumor microenvironment (TME) that plays a critical role in cancer progression and therapeutic response. Tumor-associated macrophages (TAMs) constitute a major immune cell type infiltrating the brain TME, and increasing TAM abundance is associated with more aggressive disease in human brain cancers. To gain insight into the dynamic interactions between TAMs, cancer cells, and other components of the TME during disease progression and therapeutic intervention, we have developed a multimodal longitudinal imaging strategy. We combined macroscopical magnetic resonance imaging (MRI) with subcellular resolution two-photon intravital microscopy. By using this strategy, we revealed that the migratory behavior of TAMs is different in genetically distinct glioblastomas, and in response to macrophage-targeted therapy.
We have also taken advantage of multispectral fluorine-19 MRI to noninvasively and longitudinally monitor TAMs by injecting perfluorocarbon-containing nanoparticles (PFC-NP) in preclinical models of gliomagenesis, breast-to-brain metastasis, and breast cancer. Multispectral 19F MRI with two distinct PFC-NP allowed us to identify spatially and temporally distinct TAM niches in radiotherapy-recurrent gliomas. These studies highlight the power of multimodal imaging and 19F MRI as valuable tools for the non-invasive and longitudinal monitoring of the TME in cancer. Together, our results underscore the importance of studying cancer longitudinally in an in vivo setting, to reveal complex and dynamic alterations in the TME during disease progression and therapeutic intervention.

Session 2: Multimodal integration

Laura Mezzanotte, Erasmus MC, Netherlands.

Multiscale and multimodal imaging of cancer using novel bioluminescent tools

Bioluminescence imaging is nowadays routinely employed in biomedical and pharmaceutical research to study gene expression and protein interactions, and to assess drug efficacy and other interventions for cancer. The main advantage of bioluminescence imaging relies on the high signal to noise ratio, generating high specificity. In the last decades, a great scientific effort has led to generation of a plethora of bioluminescent systems aimed at improved sensitivity, resolution and multiplexing capabilities.

This lecture will discuss recent advances from my laboratory on bioluminescence microscopy imaging of single cancer cells and immune cells on chips , on multiplexed bioluminescence imaging for small and large animals and its combination with other imaging modalities (e.g. PET/SPECT, MRI; and photoacoustic imaging) to elucidate interactions between tumor and immune cells of the stroma.

Session 2: Multimodal integration

Margret Schottelius, CHUV, Switzerland.

The power of nuclear imaging in immuno-oncology

Cancer immunotherapy, i.e. the concept of enhancing tumor-specific immunity via e.g. adoptive T-cell transfer, immune checkpoint inhibition or other interventions, has evolved as a powerful therapeutic approach in clinical oncology. However, durable clinical responses are only achieved in a relatively small fraction of patients (on average 30%), while the majority of patients either do not respond to immunotherapy or develop resistance.

Mechanisms underlying resistance or short-term response to these therapies are intricate and dynamic, and a more detailed and accurate insight into the highly complex and interwoven mutual relationships between the tumor, the cells of the tumor microenvironment (TME) and the immune system are required to identify reliable predictive biomarkers for ICI therapies. These are urgently needed for enhanced predictability of therapy response vs non-response and thus improved patient selection.

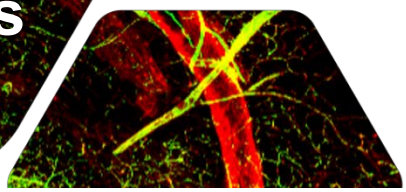
The complexity of the interplay of the tumor with its immune microenvironment, its individual kinetics, the multitude of distinct cell types contributing to or preventing efficient therapy provides a huge abundance of potential molecular biomarkers, present on both tumor cells and cells of the TME. Consequently, the clinically established and newly emerging nuclear imaging concepts in the context of immuno-oncology cover a broad scope, including:

- in vivo quantification of the expression therapeutic targets (PD-1/PD-L1/CTLA-4),
- pre- and post-therapeutic assessment of the tumor immune status by quantitative imaging of specific immune cell populations (CD8+ T cells, CD4+ T cells, neutrophils, NK cells),
- quantification of pro-tumorigenic immune cell infiltrates (e.g. M2 macrophages)
- imaging of specific pro-tumorigenic cells of the TME, e.g. FAP+ CAFs, and
- assessment of the T-cell activation status by targeting specific activation markers,

to name only the most important approaches. The true challenge now lies in the identification, selection and maybe even combination of the clinically most relevant and robust targets for nuclear imaging in the context of immunotherapy.

It will be the objective of this contribution to provide a comprehensive overview over these highly diverse nuclear imaging approaches, encompassing the full range of preclinical proof-of-concept- up to clinical studies. Additionally, it will highlight recent radiopharmaceutical developments, discuss the challenges and limitations for radiopharmaceutical development in the specific domain of TME imaging, and to weigh the potential of the respective targeting strategy to yield patient-specific, therapy-decisive and predictive information.

Session 3: Image analysis AI



Adrien Depeursinge, HES-SO Valais / CHUV, Switzerland.

Multimodal image analysis using AI for precision oncology: an overview

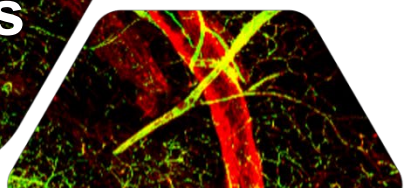
Medical images have evolved into extremely rich and complex scientific data. However, human observers are suboptimal at performing quantitative, comprehensive, and reproducible analysis of the latter.

In the past decade, Artificial Intelligence (AI) and in particular deep learning has revolutionized the ability to incorporate and linearize vast amounts of multimodal and multidimensional data in order to produce clinically actionable outputs under the form of scores, risks, decisions or regions of interest.

While focusing on multimodal clinical imaging at the macroscopic radiological scale, we will introduce the fundamentals of AI, deep learning and radiomics methods as well as their current clinical certification status. Main addressed tasks and related maturity status will be discussed, separating the expected impact and requirements of image-based AI in oncology research from practice.

Selected current efforts and platforms bred in a CHUV/HES-SO ecosystem will be presented to concretely illustrate the aforementioned methods, highlight remaining challenges and propose avenues for addressing them.

Session 3: Image analysis AI

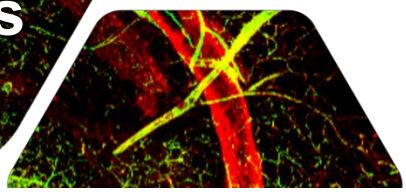


Kuangyu Shi, University of Bern, Switzerland.

Quantitative analysis of molecular imaging for the interpretation of underlying physiology

Molecular imaging provides a noninvasive way to visualize physiological features and PET is a widely applied molecular imaging method in clinical routine. With the advancement of targeted PET tracers, it is possible to assess multiple physiological features such as perfusion, glycolysis, proliferation and hypoxia. However, it is not straightforward to interpret the PET imaging signals for underlying physiology due to heterogeneous tracer delivery and uptake in the tumour microenvironment. This talk will discuss the potential of pharmacokinetic modelling, computational biology and artificial intelligence in the quantitative interpretation of the underlying physiology of PET imaging. Furthermore, it will also discuss new experimental platforms including on-chip PET and intravital multimodal imaging to assist in the development of precise quantification of PET imaging. The combination of experimental and computational methodologies may enhance the physiological interpretation of molecular imaging.

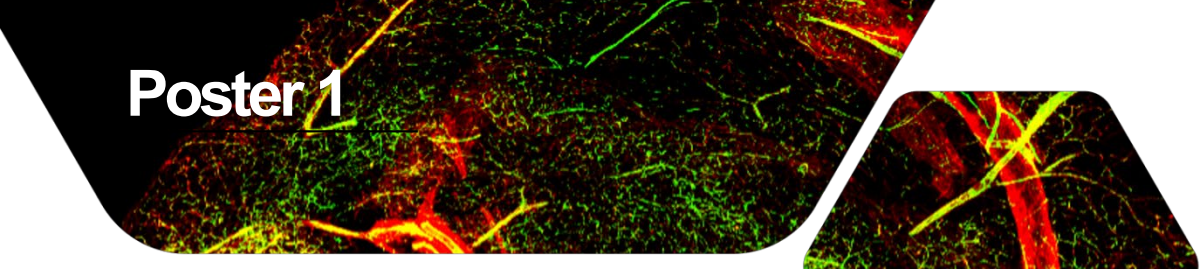
Session 3: Image analysis AI



González Santiago, Institute for Research in Biomedicine, Switzerland.

IMMUNEMAP, an open intravital microscopy imaging platform to enable Spatial-Temporal Dynamic studies in Immunology.

In vivo imaging technologies, such as intravital 2-photon microscopy (IV-2PM), allows studying immune cell behavior, unraveling unprecedented details on the spatio-temporal dynamics of the immune response. IV-2PM generates multidimensional data (3d videos with multiple acquisition channels that contain a remarkable amount of information regarding the function of the immune response. IV-2PM videos are classically analyzed by performing cell tracking and by computing measures of cell motility and interaction. These data are typically kept in local repositories at the research institutions, which are poorly interoperable and non accessible for the community. Conversely, a growing demand for publicly available microscopy data exists, to ensure reproducibility of the experiments and to perform large-scale investigations by aggregating data from multiple laboratories. Hence, the lack of publicly available IV-2PM data hampers the application of novel data-mining methods for immunological research. Here we propose IMMUNEMAP, a cloud-based platform to store, retrieve, and analyze IV-2PM videos of immune cells. IMMUNEMAP fosters the application of the FAIR principles for open data research, maximizing data re-usage. To date, IMMUNEMAP includes more than 400 videos from broad experimental settings (inflammation, cancer, infection, steady-state), and more than 15000 single-cell tracks. We provide example applications to identify different migration patterns and analyze the effect of different imaging protocols on cell motility. In conclusion, IMMUNEMAP bridges researchers from immunology and computer science fields, fostering an interdisciplinary approach in biomedical research.



DEFINING THE ENTRY ROUTES OF ENCEPHALITOGENIC TH17 CELLS FROM THE CHOROID PLEXUS INTO THE CENTRAL NERVOUS SYSTEM IN NEUROINFLAMMATION

Amandine Brenna[1], Josephine Mapunda[1], Elisa Bouillet[1], Marta Girona Alarcón[2], Urban Deutsch[1], Gaby Enzmann[1], Britta Bausch[2], Irene Spera[1], Willy Kuo[2], Griffin Rodgers[3], Christine Tanner[3], Mattia Humbel[3], Bert Müller[3], Steven Proulx[1], Vartan Kurtcuoglu[2], Britta Engelhardt[1]

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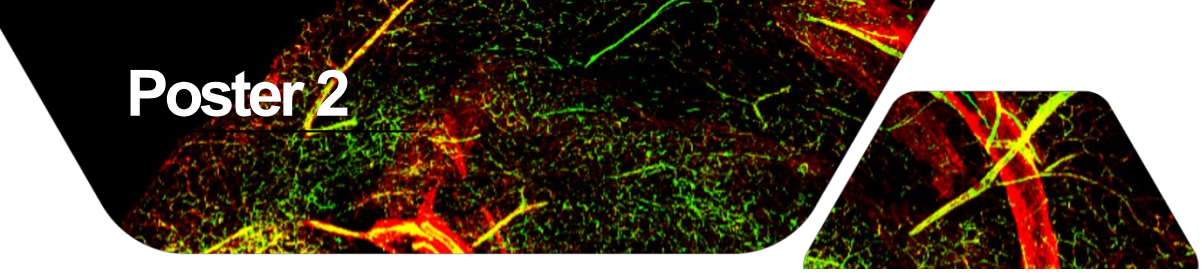
Anatomy of the choroid plexus; Blood-cerebrospinal fluid barrier (BCSFB); Experimental autoimmune encephalomyelitis (EAE)

The choroid plexus (ChP) is a highly vascularized tissue located in each ventricle of the brain. The ChP stroma is surrounded by a monolayer of cuboidal epithelial cells resting on a basal lamina that form a blood-cerebrospinal fluid barrier (BCSFB). The ChP is secreting CSF and was suggested to provide an alternative CNS entry site for encephalitogenic Th17 cells in the context of multiple sclerosis (MS). The overall goal of this project is to define the precise anatomical entry routes of encephalitogenic Th17 cells from the ChP stroma into the CNS. We make use of experimental autoimmune encephalitis (EAE), an animal model of MS, induced by the adoptive transfer of in vitro polarized encephalitogenic Th17 cells into novel syngeneic fluorescent blood-brain and BCSF barrier reporter mice. Th17 cell trafficking through the ChP is studied by combining *in vivo* imaging approaches including two-photon intravital microscopy (2P-IVM), light-sheet microscopy and *in vivo* synchrotron radiation-based micro- computed tomography (SRμCT). Employing SRμCT, we are in the process of identifying the precise 3D anatomy of the ChP. Adoptive transfer of fluorescently labeled Th17 cells into syngeneic fluorescent brain barrier reporter mice allows to visualize their trafficking through the ChP.

Making use of the established models we will identify the precise anatomical pathways of Th17 cell entry into the CNS via the ChP during health and EAE.



Dendritic cells direct circadian anti-tumor immune responses

Chen Wang[1], Coline Barnoud[1], Burak Kizil[1], Christoph Scheiermann[1,2]

[1]*Department of Pathology and Immunology, Faculty of Medicine, University of Geneva, Geneva, Switzerland*

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Keywords
Circadian, cancer immunology, vaccination

Background

The process of cancer immunosurveillance is a mechanism of tumor suppression that can protect the host from cancer development throughout its lifetime. Yet, it is unknown whether its effectiveness fluctuates over a single day. Here, we demonstrate that the initial time-of-day of tumor engraftment dictates ensuing tumor growth across murine cancer models.

Methods

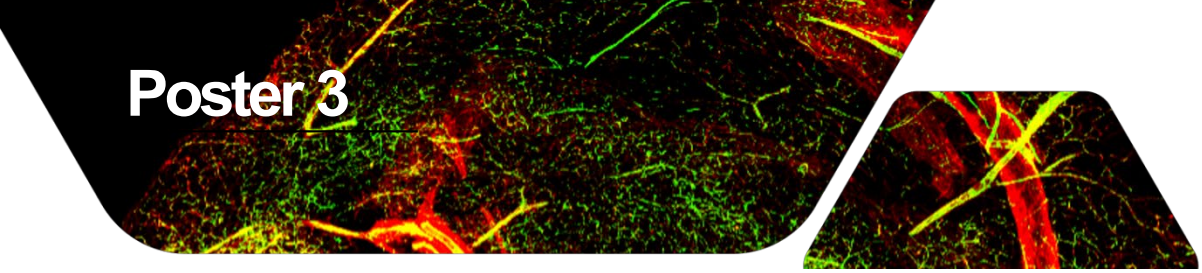
In this study, we use mouse melanoma, colon cancer, and breast cancer models to study time-of-day-dependent anti-tumor immune responses. We use immunodeficient mice and animals lacking lineage-specific circadian functions to explore the effect of immune system. We perform similar experiments using human immune cells and retrospectively analyze human vaccination data to study circadian anti-tumor effects in humans.

Results

Using immunodeficient mice and animals lacking lineage-specific circadian functions, we show that dendritic cells (DCs) and CD8+ T cells exert circadian anti-tumor functions that control melanoma growth. Specifically, we find that rhythmic trafficking of DCs to the tumor draining lymph node (dLN) governs a circadian response of tumor antigen-specific CD8+ T cells, which is dependent on circadian expression of the co-stimulatory molecule CD80. Consequently, cancer immunotherapy is more effective when synchronized with DC functions and shows circadian outcomes in both mice and humans.

Conclusion

These data demonstrate that circadian rhythms of anti-tumor immune components are not only critical for the control of tumor growth but can also be exploited therapeutically.



Deciphering the role of Sec22b contact site tethering in antigen cross-presentation

Maria Cruz Cobo, Marion Mandavit, Nina Criado Santos, Farah Mansour, Paula Nunes-Hasler

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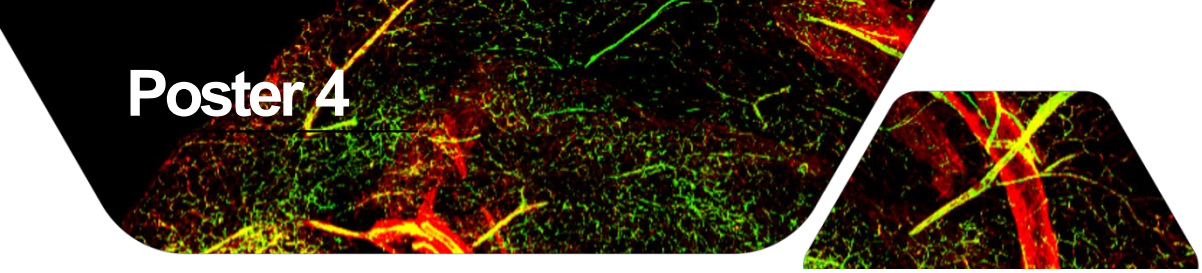
Antigen cross-presentation is essential for immunity against intracellular pathogens and cancer, however its mechanisms remain poorly understood. Phagocytosis potentiates cross-presentation, though why is unclear. Sec22b is thought to promote cross-presentation by mediating fusion of ER-to-Golgi Intermediate Compartment (ERGIC) vesicles, delivering ER-resident MHC-I peptide-loading complexes to phagosomes, yet this was never directly shown. Using 3D correlation light-electron microscopy we found that Sec22b localizes to and regulates non-fusogenic structures associated with non-vesicular lipid transfer, called ER-phagosome membrane contact sites. Sec22b knockdown reduced contacts, increased phagosomal PI(4)P and increased phago-lysosomal fusion. This phenotype was rescued by increasing tethering or overexpressing the wild-type but not mutant lipid exchange protein ORP8. Since mild phago-lysosome fusion is critical for antigen processing, we assessed the impact on cross presentation using JAWS dendritic cells. Surprisingly, in B3Z CD8 T cell co-cultures, though phago-lysosome fusion partly correlated with levels of MHC-I-peptide complexes, this did not translate to correlation with T cell activation. Instead, unexpected Sec22b-dependence of baseline levels MHC-I as well as IL-1β secretion may explain the discrepancy. Future studies will confirm these results and determine whether Sec22b-dependent cytokine secretion is related to contact-site mediated membrane repair, and whether ERGIC fusion truly occurs. Such studies may reveal novel molecular targets of therapeutic interest.



A CHEMOKINE-DRIVEN FEEDBACK LOOP PREVENTS ABERRANT T CELL ACTIVATION/ OVERSTIMULATION BY TERMINATING THEIR CROSSTALK WITH DENDRITIC CELLS

Lukas M. Altenburger[1], Daniela Claudino Carvoeiro[1], Jianwen Zhou[2], Philippe Dehio[3], Caroline Krüger[4], David Francisco[5], Laura Chiara[4], Petra Pfenninger[1], Jose Martínez Magdaleno[1], Jun Abe[1], Rémy Bruggmann[5], Jörn Dengjel[2], Matthias Mehling[3], Matteo Iannacone[4,6,7], Jens V. Stein[1,*]

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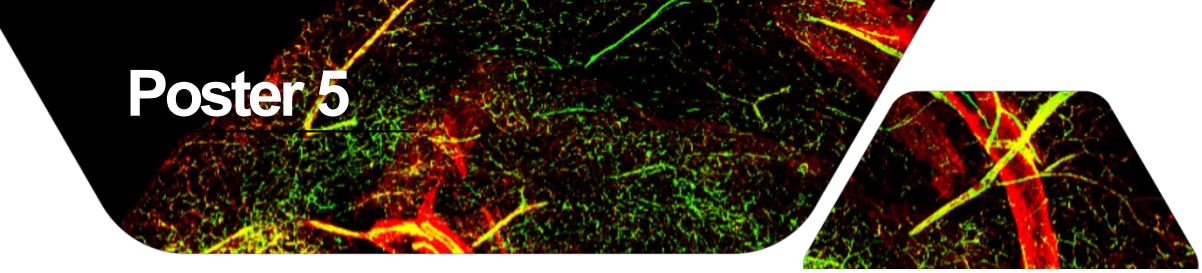
• Corresponding author

Abstract

CCR7 mediates the colocalization of activated dendritic cells (DCs) and naïve T cells (T_N) in the paracortex of lymphoid organs, where its ligands CCL19 and CCL21 are produced by local fibroblasts. This process has evolved to shorten the time span required for clonal selection and expansion of $CD8^+$ effector T cells (T_{EFF}) by increasing the likelihood for T_N to find and engage with cognate pMHC-presenting DCs. Here, we describe a novel function for CCR7 on $CD8^+$ T cells in regulating T_{EFF} generation. In addition to its role in facilitating T cell-DC encounters, CCR7 promotes T cell disengagement from DCs at late stages of cognate interactions. Mechanistically, CCR7 signals drive the promigratory Rac activator DOCK2 away from the late T cell-DC interface to induce T cell detachment. CCR7-mediated T cell uncoupling from DCs correlates with rapid onset of proliferation and differentiation of T_{EFF} characterized by high effector function and low PD1 expression. In turn, absence of a CCR7 “rheostat” causes protracted T cell-DC interactions and TCR signal integration, yielding dysfunctional T_{EFF} that share hallmarks with precursors of exhausted T cells including high PD-1 expression and reduced *in vivo* persistence. In sum, our results shed light on the physiological control of TCR signal duration and identify a key role for lymphoid tissue-expressed CCR7 ligands to prevent dysfunctional T cell activation, with potential ramnifications for the design of *in vitro* T cell activation protocols used in adoptive cell therapy.



VE-CADHERIN IDENTIFIES ARACHNOID AND PIA MATER CELLS: A MISSING LANDMARK FOR *IN VIVO* IMAGING OF THE IMMUNE SYSTEM AT THE CNS BORDERS

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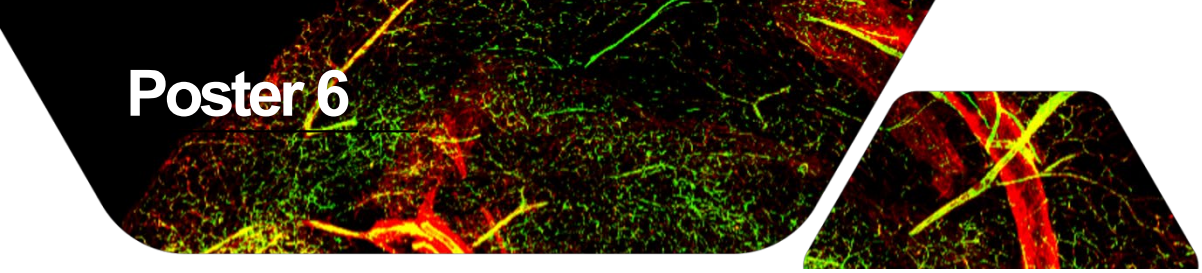
Keywords:

two-photon imaging, leptomeninges, CNS immunity

Abstract

The meninges at the outer border of the central nervous system (CNS) contribute to maintaining CNS protection and immunity. How barrier properties of the leptomeningeal layers control access of immune cells and immune mediators into the CNS is however not well understood. Here we show junctional localization of VE-cadherin in arachnoid and pia mater cells bordering the cerebrospinal fluid (CSF) filled subarachnoid space (SAS). In vivo imaging of the CNS in VE-cadherin-GFP knock-in mice allowed for direct observation of arachnoid and pia mater barrier properties to CSF derived tracers and T cells during health and neuroinflammation. Here we show that pia mater is not a true barrier for small molecular size tracers during health and neuroinflammation. Transfer of fluorescent CD8 T cells into VE-cadherin-GFP knock-in mice allowed for direct observation of the interaction between CD8 T cells and pia mater fibroblasts, revealing the role of ICAM-1 and VCAM-1 mediating the adhesion and crawling of CD8 T cells on the pia mater under neuroinflammation. Taken together, we have identified VE-cadherin as a novel landmark for *in vivo* imaging of the leptomeninges as a prerequisite to visualizing the leptomeningeal barrier properties controlling access of immune mediators and immune cells into the CNS during health and neuroinflammation.



Targeting HIF-2α as a strategy to overcome glioma immunosuppression.

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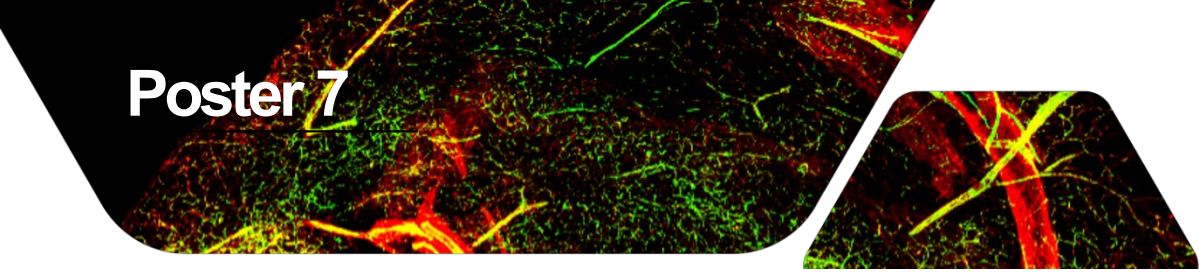
Key Words: TAMs, tumor microenvironment, HIF-2.

The glioblastoma (GBM) microenvironment is highly heterogeneous, with an immune infiltrate dominated by tumor-associated microglia/macrophages (TAMs). Their presence is associated with resistance to chemo and radiotherapy and an impediment to successful immunotherapy. Hypoxia, a key poor prognosis feature in GBM, can regulate macrophage pro-tumoral polarization through the expression of hypoxia-inducible factor 2α (HIF-2). Specifically, the lack of HIF-2 in the myeloid lineage resulted in decreased TAM infiltration and tumor progression in colon, liver, and breast tumor models, but its importance in the polarization and function of microglia and macrophages in the GBM microenvironment is still unknown. Here, we report the first steps in elucidating this issue. We show that HIF-2 inhibitor (PT2385) administration increased overall survival and decreased tumor growth in GL261 tumor-bearing mice. Moreover, PT2385 regulated TAM infiltration and modulated T cell activation at the end-term point of mice survival and at the mid-term of tumor progression. We analyze HIF-2 contribution to microglia polarization, HIF-2 expression was increased in M2-like microglia and its inhibition prevented M2-like polarization, without modifying M1-like polarization. Our findings suggest that HIF-2 regulation of microglia/macrophage polarization could be one of the possible mechanisms involved in the immunosuppression of the GBM tumor microenvironment. Overall, our results support HIF-2 targeting as a rational therapeutic opportunity for GBM and suggest TAMs as an encouraging targetable cell population. This encourages us to elucidate the mechanisms by which HIF-2 targeting could shape the tumor microenvironment to overcome GBM immunosuppression.



HEAD-TO-HEAD COMPARISON OF DIFFERENT CLASSES OF FAP RADIOLIGANDS DESIGNED TO INCREASE TUMOR RESIDENCE TIME: MONOMER, DIMER, ALBUMIN BINDERS, AND SMALL MOLECULES *VS* PEPTIDES

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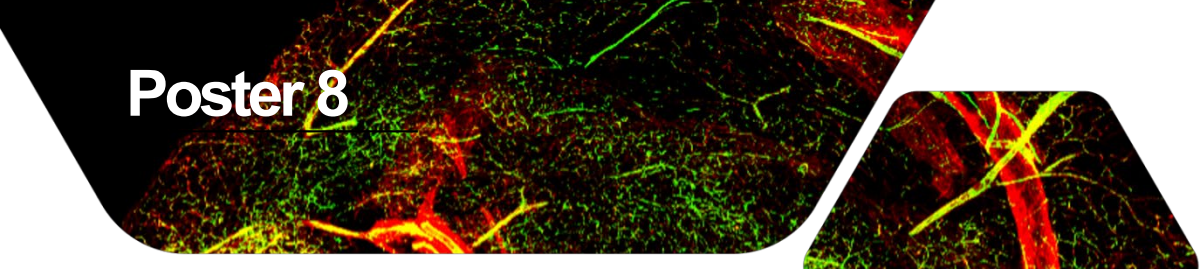
Key words
Research topic: targeted radioligand **Method**: SPECT-CT and biodistribution **Application of findings**: Oncology

Study relevance, rationale and hypothesis: Radioligands targeting Fibroblast Activation Protein-α (FAP) have demonstrated high oncological diagnostic potential. However, their therapeutic potential is impaired by the short tumor residence time. Several strategies have been tested to overcome this limitation. So far, a head-to-head comparison of these strategies has never been done. In this work, we compared the monomer FAPI-46 *versus* a) a dimeric version (FAPI-46-F1D) b) two albumin-binders-conjugates (FAPI-46-Ibu and FAPI-46-EB), and c) the cyclic peptide FAP-2286. Our aim was to identify the strengths and limitations of all strategies.

Methods: LogD, IC_{50} and *in vitro* characterization of all ligands were evaluated. *In vivo* SPECT/CT and biodistribution studies were conducted in FAP-positive and FAP-negative tumor-bearing mice (dual model with different FAP-expression). Areas under the curve (AUC) of the tumor uptake and the tumor-to-critical-organs ratios were assessed.

Results: All radioligands showed picomolar IC_{50} values. ^{177}Lu-FAPI-46-based radioligands showed similar uptake between the two tumor models, which was different compared to the peptide. *In vivo*, ^{177}Lu-FAP-2286 showed the cleanest background among all. The AUC of the tumor uptake was higher for ^{177}Lu-FAPI-46-F1D and ^{177}Lu-FAPI-46-EB in HT1080.hFAP xenografts and for ^{177}Lu-FAPI-46-EB and ^{177}Lu-FAP-2286 in HEK293.hFAP xenografts. AUC of the tumor-to-critical-organs ratios were in favor of ^{177}Lu-FAP-2286, except from tumor-to-kidneys.

Conclusion: Dimerization and the peptide *vs* small molecules are the most promising strategies for prolonging tumor residence time. The peptide showed better tumor-to-background ratios, besides tumor- to-kidneys, but its tumor uptake was FAP-expression-dependent. The outcome of the albumin-binder strategy depended heavily on the albumin-binding moiety.



A PERMANENT THORACIC WINDOW MODEL FOR INTRAVITAL IMAGING OF NON-SMALL CELL LUNG CANCER

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Abstract

Objectives

Intravital microscopy is a powerful approach allowing real-time observation of living animal tissue at a high resolution. The imaging of lungs is challenging and only few experimental thoracic devices exist. However, most of them are not designed for repeated imaging and do not allow longitudinal observation. Here, we aimed to optimize a permanent thoracic window mouse model adapted for upright 2-photons microscope that enables long-term intravital imaging of non-small cell lung cancer (NSCLC) tumors and surrounding healthy lung tissue.

Methods

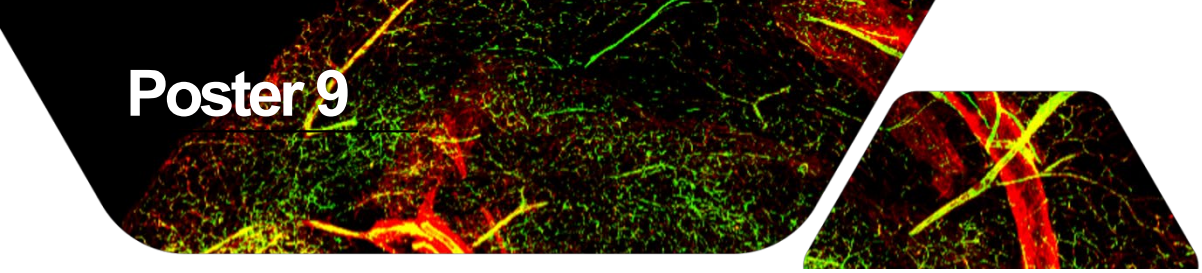
Our model is based on a previously published window model designed for inverted 2-photons microscope. The design and dimension of the window frame were modified to fit with an upright 2-photon microscope and ameliorate the stabilization of the device. Moreover a trigger module was used to synchronize image acquisition with breathing movements. Different approaches including chunk graft, intravenous and intrathoracic inoculation of NSCLC-derived cells were tested to implant a tumor underneath the observation window. Finally, Rhodamine 6G and Pacific Blue dextran were injected intraperitonally and intravenously respectively for leukocytes and blood vessels visualization.

Results

The modified thoracic window could be successfully implanted and kept for a duration of 6 weeks without affecting the well-being of the animals. Intravital image acquisition of vessels and leukocytes trafficking was possible in healthy lung tissue. Some challenges remain to get a functional tumor underneath the window.

Conclusion

If successful, this unique thoracic window could open new perspectives for the understanding of NSCLC tumor microenvironment, therapy-induced modulation and therapy refinement.



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

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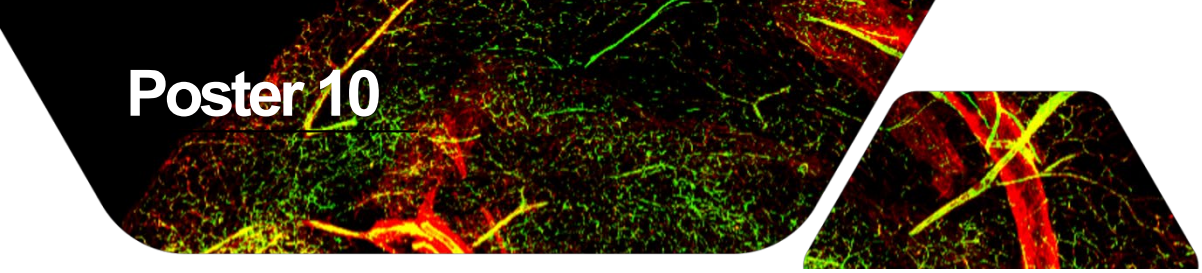
Neutrophils are innate immune cells known for their ability to fight pathogens. Their trafficking throughout the body is key to perform their functions. However, the mechanisms of neutrophil trafficking to lymph nodes are not fully clear. Using a murine model of dermal infection with *Leishmania* parasites,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



TIME-OF-DAY DEPENDENT CHANGES IN CHEMOKINE RECEPTOR EXPRESSION DICTATE TRAFFICKING PROPERTIES OF HUMAN LEUKOCYTES

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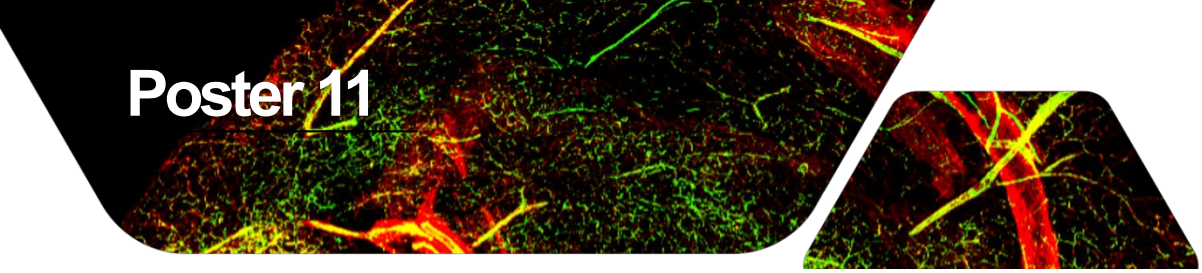
Keywords: leukocyte trafficking, image cytometry, circadian rhythm

Human leukocyte blood counts are regulated by a concerted cycle of egress from bone marrow into blood and successive extravasation into organs. This recruitment process crucially depends on chemokine receptors, which regulate leukocyte activation and mediate chemotactic responses. Here, we employed a 3D chemotaxis assay to evaluate the migration of all leukocyte subtypes isolated from blood of multiple donors along various chemokine gradients *in vitro* at different time points of the day. More than 30 single cell motility and morphological parameters were acquired by 4D live imaging using spinning-disk confocal microscopy and were subsequently analyzed utilizing functional UMAPs (Uniform Manifold Approximation and Projection for Dimension Reduction) for cluster identification. The screening of the surface proteome of individual cells was performed by multicolor flow cytometry to examine a potential correlation between time-of-day dependent differences in migratory behavior and corresponding changes in chemokine receptor expression level. Thus, this experimental pipeline enables the functional profiling of leukocytes at the single cell level and allows the identification of functional leukocyte clusters by linking time-controlled chemokine receptor expression to the migratory response.



NOVEL RADIOHYBRID PET-TRACERS FOR THE IMAGING OF NEUROENDOCRINE TUMORS

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Key words

PET Tracer, Neuroendocrine Tumors, Radiohybrid

Abstract

Radiohybrids (rh) [1] provide molecular twins by SiFA-based ^{18}F-labeling or radiometal-chelation of corresponding precursors. This provides theranostic companion tracers and allows the free choice of radionuclide (^{18}F or ^{68}Ga). To exploit these features for SST_2-targeted PET imaging of neuroendocrine tumors, we investigated DOTATATE-based rh analogs, using the DOTA-moiety as a bridging unit.

Compounds were synthesized and characterized via RP-HPLC and ESI$^+$-MS. Human serum albumin (HSA) binding was determined using a HSA-HPLC column. The hydrophilicity (logD) of the ^{nat}Ga/^{18}F-labeled compounds was assessed [2]. SST_2-affinities were determined using CHO_{hSST2}-cells and [^{125}I]I-TOC. Internalization studies (1h) were carried out in AR42J cells, using [^{125}I]I-TOC and [^{18}F]SiFA*lin*-TATE as references. For biodistribution studies, female AR42J xenograft bearing CD1 nu/nu mice were used.

[^{18}F][Ga]SSA2.2 (D-Glu-D-Cit-D-Cit-D-Dap-(N-SiFAlin-*N,N*-Me_2-Gly)-D-Lys(trans-[Ga]DOTA-TATE)) and [^{18}F][Ga]SSA2.5 (D-Glu-D-Glu-D-Glu-D-Dap-(N-SiFAlin-*N,N*-Me_2-Gly)-D-Lys(trans-[Ga]DOTA-TATE)) have identical logD (-2.3±0.04), high SST_2-affinity (3.3±0.5 and 2.8±0.2 nM, respectively SiFA*lin*-TATE: 6.98±0.61) and efficient internalization ([^{18}F][Ga]SSA2.2: 234%, [^{18}F][Ga]SSA2.5: 151% of [^{18}F]SiFA*lin*-TATE).

[^{18}F][Ga]SSA2.2 and [^{18}F][Ga]SSA2.5 display high accumulation in SST_2-expressing tissues (pancreas, stomach, adrenals, tumor). With a HSA-binding of 94.3% ([^{18}F][Ga]SSA2.5: 84.8%), [^{18}F][Ga]SSA2.2 had delayed blood clearance, higher background activity, and tumor accumulation than [^{18}F][Ga]SSA2.5 and [^{18}F]SiFA*lin*-TATE (27.9±4.8 vs 18.6±6.2 and 22.7±2.7%iD/g, respectively). For [^{18}F][Ga]SSA2.2 and [^{18}F][Ga]SSA2.5, tumor/background ratios were comparable (kidney, intestestine) or superior to [^{18}F]SiFA*lin*-TATE (tumor/blood: 72 and 58 vs 28, tumor/muscle: 460 and 420 vs 150, tumor/liver: 11 and 47 vs 12).

This demonstrates for the first time the feasibility of a SiFA*lin*-based rh design using DOTA as a bridging unit and provides tracers with improved preclinical performance compared to the current standard [^{18}F]SiFA*lin*-TATE.

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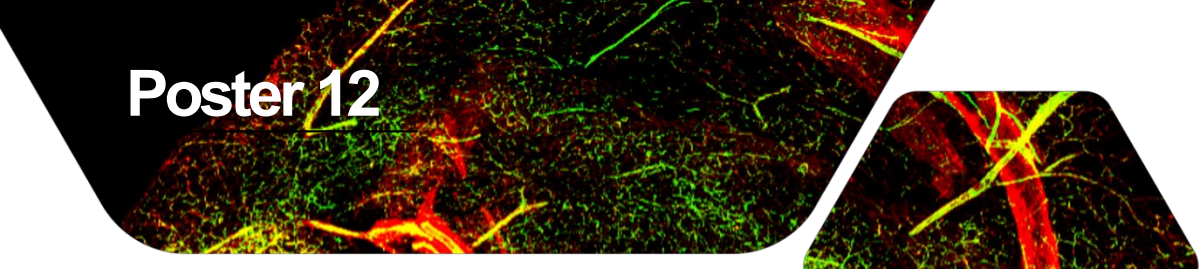

MULTISCALE IMAGING TO STUDY IMMUNOTHERAPY OF CANCER BONE METASTASIS

Martina Giampetraglia[1,2], Max Zimmermann[1,2], Gina Dunkel[1,2], and Bettina Weigelin[1,2]

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Keywords: Multiscale imaging, bone metastasis, immunotherapy

Macroscopic imaging modalities are essential clinical tools for diagnosis and treatment monitoring and are frequently used in preclinical research to evaluate new therapeutic strategies. While macroscale imaging techniques, such as PET, MR and CT, provide systemic anatomical and physiological information, each image voxel integrates signals from hundreds to thousands of cells, masking the underlying heterogeneity in single-cell behavior. Combined microscopic and macroscopic imaging is therefore required to capture physiological processes at different biological levels.

The bone cavity in mice is typically too small to be efficiently sampled by the large voxels acquired in macroscopic imaging, such as PET and functional MR. The bone marrow of long bones is further inaccessible by microscopic imaging techniques due to light scattering in the dense cortical bone. Studying cellular processes, such as cancer bone metastasis and (immune)therapy response longitudinally by non-invasive imaging in bone is therefore currently impossible.

We here show the development of a tissue-engineered bone model which creates a bone marrow cavity with a large enough volume to enable macroscopic imaging in immunocompetent mice. The engineered bone ossicle uniquely enables combining functional MR, light-sheet microscopy, intravital microscopy and highly-multiplexed tissue analysis. The workflow will be used to study tumor growth, vascular perfusion and immune infiltration to test combination therapies for optimized immunotargeting of prostate cancer in bone.