DKTK Freiburg Scientific Community Meeting

Monday 7 July 2025 - Tuesday 8 July 2025

Caritas Tagungszentrum Freiburg

Book of Abstracts

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Pre-clinical therapeutic target identification for renal and bladder cancers

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Clear cell renal cell carcinomas (ccRCC) are partly responsive to immune checkpoint inhibitor therapies that are based on inhibition of PD-1 and CTLA-4. We have described an autochthonous mouse model of ccRCC that is resistant to immune checkpoint inhibitor therapies and have identified numerous candidate immunosuppressive molecular pathways in ccRCC cells and in infiltrating myeloid lineage cells. Our ongoing work aims to systematically inhibit these pathways to identify the most promising approaches of improving anti-tumour immunity to guide the development of the next generation of immune-based ccRCC therapies.

Bladder urothelial carcinomas are characterised by recurrent mutations in several epigenetic tumour suppressor genes, highlighting that altered cellular epigenetics is a fundamental driver of these tumours. The UcarE Forschungsgruppe seeks to exploit epigenetic vulnerabilities of urothelial carcinomas though pharmacological and genetic screenings, genetic engineering of mouse and human cellular models and through pre-clinical testing of epigenetic drugs using human organoid and slice culture systems.

Preferred type of presentation:

4

Extracorporeal Photopheresis resolves immune checkpoint inhibitor associated colitis through local adiponectin induction

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The development of immune-related adverse events (irAEs) in cancer patients receiving immune checkpoint inhibitors (ICIs) cause morbidity, necessitates treatment cessation and limits ICI efficacy. Comparing different first- and second-line irAE treatments, we found that glucocorticosteroids, TNFα blockade, and α4β7-integrin inhibition reduced anti-tumor immunity in mice. We compared these therapies against extracorporeal photopheresis (ECP) and found that ECP has no negative effect on anti-tumor immunity in multiple preclinical tumor models. Based on these findings, we tested ECP in different ICI-colitis models and observed significantly reduced colitis severity after treatment. Mechanistically, we identified that ECP-treated splenocytes accumulate specifically in

the inflamed intestinal tract, but not the tumor microenvironment. Apoptotic splenocytes were engulfed by intestinal phagocytes, which rendered these towards an anti-inflammatory phenotype. Immunosuppressive macrophages secreted adiponectin to resolve inflammation in the intestinal tract. Local adiponectin production elicited a tissue-specific effect by reducing pro-inflammatory tissue-resident memory T-cell activation, CD4+IFN-γ+ T-cells and inflammatory myeloid cells, while sparing tumor-specific T-cell development.
Following our preclinical investigations, we tested ECP in a prospective phase-Ib/II trial (EudraCT-No.2021-002073-26) with 30 patients and found low ECP-related toxicity. At week 12 of therapy, the overall response rate (ORR) for all irAEs was 96%; the ORR for colitis was 100%. The colitis-specific complete remission rate was 93%. Glucocorticosteroids could be reduced for all patients after ECP-therapy. The ECP-adiponectin axis reduced intestinal activation in patients with ICI-colitis without evidence of loss of anti-tumor immunity.
In conclusion, we identified ECP-induced adiponectin as an immunomodulatory mechanism to control ICI-induced inflammation without blocking anti-tumor immunity.

Preferred type of presentation:

5

CD73 and MEK Inhibition improve T Cell function in NRAS-Mutant AML: A Strategy for Post-Transplant Immune Surveillance

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Relapse after allogeneic hematopoietic stem cell transplantation (allo-HCT) remains a major obstacle in the treatment of acute myeloid leukemia (AML), often driven by immune escape mechanisms. NRAS mutations, found in ~12% of AML cases, activate RAS-MAPK signaling and are frequently acquired late in disease progression, suggesting a role in immune evasion and post-transplant relapse.
Using a syngeneic transplantation mouse model with NRASG12D -transduced hematopoietic stem cells (HSCs), we show that NRAS activation upregulates CD73 on myeloid cells and suppresses T cell function—marked by reduced TNF-α, impaired $CD4^+$/$CD8^+$ proliferation, and increased Tregs. These immunosuppressive effects were largely reversed upon transplantation of CD73-deficient, NRAS-transduced cells, which also exhibited increased MHC class II expression
In an allogeneic model, NRAS-driven leukemia induced similar T cell dysfunction, including reduced effector proliferation and cytokine production. CD73 inhibition restored T cell effector function, increased granzyme B, enhanced memory differentiation, and further upregulated MHC-II.
MEK inhibition (Trametinib) reduced CD73 expression, enhanced T cell proliferation, and improved leukemia control in a recall immunity experiment, suggesting durable anti-leukemic memory.
Our findings identify CD73 as a central immune checkpoint in NRAS-mutant AML. Targeting CD73 and MEK represents a promising strategy to enhance post-transplant immune surveillance and prevent relapse

Preferred type of presentation:

6

Oncogenic signaling promotes the expression of CD155 in acute myeloid leukemia facilitating immune escape

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Allogeneic hematopoietic stem cell transplantation (allo-HCT) is the only curative therapeutic option for high-risk acute myeloid leukemia (AML) patients. The success of allo-HCT relies on the so-called graft-versus-leukemia (GVL) effect. This term defines the immunological process in which residual leukemic cells are eliminated by donor immune cells. However, more than 30 % of AML patients relapse after allo-HCT due to leukemic cells escaping immunosurveillance, for example, by expressing immune checkpoint ligands.

The immune checkpoint molecule CD155, also known as polio virus receptor, is highly expressed on AML blasts, and previous studies have shown that high expression of CD155 is correlated with higher relapse rates post allo-HCT.

In this study, we investigated the role of CD155 in the GVL effect and identified the oncogenic signaling pathways that regulate CD155 expression. Using allo-HCT mouse models, we found that the absence of CD155 on both donor immune cells and on leukemic cells is beneficial. Furthermore, blocking the immune checkpoint using an anti-CD155 antibody prolonged the survival of leukemia-bearing mice, indicating the importance of CD155 for GVL effect. By introducing oncogenic mutations commonly found in AML patients into mouse hematopoietic stem cells, we found that multiple oncogenic mutations, such as cKit-D816V and NRAS-G12D, enhance CD155 expression. Treatment with inhibitors targeting the specific oncogenic mutation or the downstream signaling pathways resulted in reduced CD155 expression.

These findings highlight the potential of targeting oncogenic pathways regulating CD155 expression as a therapeutic strategy to enhance the GVL effect and reduce relapse rates in AML patients post-allo-HCT.

Preferred type of presentation:

Poster Presentation only

7

Saturation mutational scanning uncovers druggability of all FGFR point mutations

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Variants of unknown significance represent the biggest challenge for genomics-based precision oncology making high throughput functional genomics essential to characterize them. Aberrantly activated Fibroblast Growth Factor Receptors (FGFRs) frequently drive tumorigenesis across many tumor entities. Approved selective inhibitors (FGFRis) are available. However, it remains largely unknown which of the many different FGFR point mutations are druggable, i.e. activating signaling while not mediating resistance thereby substantially limiting the therapeutic potential of approved FGFRis.

We implemented a saturation mutational scanning platform to screen all 29259 possible point mutations in FGFR1-4. In positive selection screens of the kinase domains, we already identified 474 activating and 738 resistance-mediating mutations to the FGFRis pemigatinib or futibatinib yielding 301 druggable point mutations with a strong PS3/BS3 evidence level. Mutations in the same codon could strongly differ in their impact, underlining the necessity for a saturation approach. Importantly, our functional screens identified 97% of acquired resistance mutations in a clinical trial.

In summary, we provide a comprehensive and clinically highly relevant catalog of every single druggable point mutations in FGFR which is readily available for clinical decision support.

For the future, we see the necessity to expand our dataset to the regions outside of the kinase domain, to include the third FGFR inhibitor approved in Germany, erdafitinib, as well as to develop and test a second, independent activation model to allow robust predictions of the activation and drug response impact to enable a planned clinical trial to test FGFRi treatment for tumors with FGFR point mutations.

Preferred type of presentation:

8

Distinct immune landscapes in Autoimmune vs. Checkpoint Inhibitor-Associated Hepatitis revealed by spatial profiling

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Immune-related adverse events (IRAEs) to checkpoint inhibitor therapy can be life-threatening, yet their underlying mechanisms remain poorly understood. It is often presumed that the involved cellular mechanisms bear similarities with spontaneous autoimmune diseases. In this work, we compared the intrahepatic immune landscape of checkpoint-inhibitor-associated hepatitis (ICI-Hep) with that of spontaneous autoimmune hepatitis (AIH) using spatial single-cell and spatial transcriptomic analyses of inflamed liver tissue from affected patients.
Our findings reveal fundamentally distinct immune landscapes in ICI-Hep and AIH. ICI-Hep is characterized by pronounced interactions between activated cytotoxic $CD8^+$ T cells and activated myeloid cells—features not seen in AIH, which instead shows an enrichment of exhausted and tissue-resident T cells alongside B cells. Notably, the pathognomonic immune cell interactions in ICI-Hep involve active mTOR signaling. Inhibition of mTOR led to reduced activation of $CD8^+$ and myeloid cells and resulted in clinical improvement of liver inflammation in patients.
These results highlight distinct cellular pathomechanisms in IRAEs and establish mTOR signaling trageting as a rational therapeutic option for severe ICI-Hep.

Preferred type of presentation:

Poster Presentation only

9

In Situ Peptide Identification by Parallel-Accumulation-Serial Fragmentation Supported MALDI MS/MS imaging: A Tool for Spatial Proteomics in Complex Disease Tissues

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Spatial proteomics by matrix-assisted laser desorption/ionization (MALDI) imaging enables rapid, label-free peptide analysis directly from tissue sections. However, in situ peptide identification remains a major challenge, limiting the broader utility of this technique. In this study, we present a novel workflow that integrates Trapped Ion Mobility Spectrometry-based Parallel Accumulation–Serial Fragmentation into MALDI imaging to enable multiplexed MS/MS imaging and MASCOT

peptide-to-spectrum matching for spatial peptide identification. We demonstrate the feasibility of this pipeline across three distinct samples: a tumor xenograft model, mouse kidney tissue, and an amyloidosis tissue microarray (TMA) comprising different subtypes. In each case, iprm-PASEF enabled the identification of multiple tryptic peptides in a single imaging experiment, with spatially resolved fragment ion maps and identifications corroborated by LC-MS/MS and fragment colocalization. In the TMA, we successfully identified seven amyloidosis-associated peptides, including markers from vitronectin, apolipoprotein E, and transthyretin receptor, with clear spatial correlation to Congo red-positive deposits. These three tissue types, xenograft, kidney, and amyloidosis TMA, collectively illustrate the method's applicability to resolve peptide distributions and molecular characterization from complex physiological and pathological heterogeneous tissues. These findings underscore the promise of tryptic peptide MS/MS MALDI imaging for advancing spatial proteomics in oncology and pathology research, with potential applications in tumor characterization, microenvironment profiling, and treatment response studies, particularly when integrated with complementary omics and histological approaches, though further validation across different tissue samples is needed.

Preferred type of presentation:

10

Advancing Radioproteomics: Integrating PSMA PET/CT and mpMRI with Localized Proteomic Profiling in Prostate Cancer

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Background
Current diagnostic methods for primary prostate cancer (PCa), such as image-guided biopsies, carry risks and may miss tumor heterogeneity. This has driven interest in non-invasive imaging approaches like multiparametric MRI (mpMRI) and PSMA PET/CT, which capture the full tumor burden. This study integrates radiomic features from imaging with histopathology and proteomics from distinct tumor subregions to enhance the molecular interpretation of imaging characteristics in PCa.
Methods
Twenty patients with intermediate- or high-risk PCa underwent preoperative mpMRI and PSMA PET/CT, followed by radical prostatectomy. Imaging and histopathology were co-registered for voxel-based analysis. Proteomic profiling of selected regions was performed using LC-MS/MS, enabling correlation of imaging features with localized protein expression.
Results
Analysis of 77 tumor and adjacent non-malignant tissue samples identified over 2,400 proteins. The maximal standardized uptake values (SUV max) from PSMA PET/CT correlated with PSMA expression, validating the radioproteomic approach. High SUV regions were enriched in proteins related to telomere maintenance and RNA processing, while low SUV regions were associated with extracellular matrix remodeling. MRI-derived apparent diffusion coefficient (ADC) values correlated with proteins involved in cell adhesion and Golgi organization. Distinct proteomic signatures were identified across tumor subregions, revealing potential biomarkers for non-invasive tumor characterization and risk stratification.
Conclusion
By integrating advanced imaging with proteomic profiling, this study highlights molecular correlates of imaging features in PCa. These insights support the development of imaging-based biomarkers and personalized therapeutic strategies, bridging non-invasive diagnostics with tumor biology.

Preferred type of presentation:

Poster Presentation only

11

Spatiotemporally-Resolved Single-Cell Genomics Reveals Tumor Immune Escape Mechanisms in Glioblastoma

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Our immune system possesses an intrinsic capability to recognize and eliminate malignant cells. Yet, tumors frequently subvert this defense by reprogramming tumor-infiltrating immune cells, driving immune evasion and contributing to therapy resistance. Single-cell genomics bear great potential to enhance our understanding of these processes by generating detailed molecular maps of immune cell states within the tumor microenvironment. However, the destructive nature of most single-cell genomics technologies inherently limits them to static snapshots, lacking the temporal resolution required for a causal understanding of tumor-immune interactions. To overcome this limitation, we developed a novel, temporally-resolved single-cell genomics platform that leverages fluorescent in vivo timestamping of circulating immune cells. Our methodology enables the time-resolved recording of transcriptional dynamics of an immune cell once it has been exposed to the tumor microenvironment and thereby offers the possibility to retrieve tumor-immune interactions in real-time. We extended this approach to single-cell resolved spatial transcriptomics, allowing dynamic tracking of immune cell infiltration into tumor niches across space and time. Applying this technology to

a model for glioblastoma, we uncovered dynamic patterns of immune infiltration and adaptation, revealing a previously inaccessible perspective of tumor-immune interactions. Our spatiotemporal analyses identified reported drivers of immune escape in an unbiased, data-driven manner and suggested novel therapeutic vulnerabilities that could be exploited to reinvigorate anti-tumor immunity. Together, we present a novel space- and time-resolved single cell genomics technology with tremendous potential for the rational design of novel immunotherapies.

Preferred type of presentation:

12

Base Editing Unlocks NK Cell Potential for Cancer Immunotherapy

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Natural killer (NK) cells are critical components of the innate immune system, capable of targeting tumor and virus-infected cells. Their function is regulated by a balance of activating and inhibitory receptors. Among these, NKG2A—encoded by the KLRC1 gene—recognizes the non-classical MHC molecule HLA-E on target cells, delivering inhibitory signals that suppress NK cell cytotoxicity and contribute to immune evasion by tumors. CRISPR/Cas9-mediated disruption of KLRC1 has been explored to enhance NK cell function, but its clinical application is constrained by suboptimal efficiency and risks associated with DNA double-strand breaks (DSBs), including genotoxicity. In this study, we employed adenine base editing (ABE) to achieve precise and safe KLRC1 knockout in primary human NK cells. A panel of guide RNAs (gRNAs) was tested, and one highly effective gRNA/ABE combination was selected for direct comparison with CRISPR/Cas9. ABE editing achieved >95% knockout efficiency at both genomic and protein levels without being dependent on DSBs. In contrast, Cas9 editing with the same gRNA yielded lower efficiency and detectable off-target activity. In contrast, evaluation of specificity using UCAST-Seq confirmed minimal off-target effects in ABE-edited NK cells. Functional assays confirmed complete loss of NKG2A expression and significantly enhanced cytotoxicity against HLA-E–positive tumor cells. These results highlight ABE as a highly efficient and precise platform for KLRC1 knockout in NK cells, offering a safe alternative to conventional gene editing for the development of next-generation NK cell–based immunotherapies.

Preferred type of presentation:

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Translational Radioimmunotherapy

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We previously demonstrated in preclinical and case reports that hypofractionated radiotherapy (RT) can induce tumor-specific T cells, thereby acting synergistically with immune checkpoint blockade (ICB), both locally and outside the RT field (abscopal effect). In the current funding period, we have (i) tested novel treatment combinations in mice to improve the abscopal effect, particularly T cell infiltration of abscopal tumors, e.g., by adding low-dose radiation or chemotherapy, which induce T cell-attracting chemokines; (ii) participated in clinical trials investigating the abscopal effect in metastatic melanoma/NSCLC (IRINA/PARADIGM DKTK-wide joint funding project and RadImmun-NSCLC with DKTK partners in Freiburg); (iii) investigated immune biomarkers in these and other trials. In the coming years, we aim to further improve RT/ICB combinations, particularly the abscopal effect, through additional adoptive T cell transfer, e.g., in ICB-resistant or poorly antigenic tumors. We started by adding TILs, TCR-transgenic T cells, or CD133-specific CAR T cells to RT/ICB in immunocompetent abscopal mouse models without lymphodepleting preconditioning. We are establishing methods to characterize and expand TILs from NSCLC tissue, and will assess their antitumor efficacy using patient-derived organoids (ongoing cooperation with Thoracic Surgery and FREEZE-O) and xenografts in mice +/- RT. In addition, TCR-engineered T cells will be produced. Collaborations on targeted radioligand therapy, preclinical MR and PET imaging to optimize combination therapies, and identification of neoepitopes using NGS/mass spectrometry are also planned. Our medium-term goal is to enable clinical trials using TILs or TCR-transgenic T cells to enhance the abscopal effect or for adoptive transfers with T cell-attracting RT.

Preferred type of presentation:

14

From functional imgaging to new traetment approaches in radaition oncology

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Tumor hypoxia and high cell density are a well-documented phenomenon in tumours and are key factors contributing to radioresistance. Consequently, overcoming these factors are a major challenge in modern radiation oncology.

Functional imaging using F-MISO-, FDG-PET-CT and multiparametric MRT provide non-invasive methods for detecting and quantifying tumor hypoxia and cellularity at a macroscopic level.
In a prospective, exploratory analysis conducted by us, we were able to determine the spatial distribution, intensity and progression of tumour hypoxia and cell density using sequential imaging in cancers of head and neck

In cooperation with the Karolinska Institutet we developed a model for calculating expected tumor control probability (TCP). This model forms the basis for the creation of individualized radiation plans that achieve TCPs of (theoretically) 80%/90%/95% considering the biological and visible properties of the tumour cells The planned (intratumoral) dose escalation should be delivered as precisely as possible (in terms of spatial localization regarding hypoxia dynamics) and ideally as effective as possible in restoring normoxia. For this reason, dose escalation in our project is planned as an initial "boost"at the beginning of radiochemotherapy as proposed from validated simulation models.

We are presenting the outlines of the Phase-I HIBERNATE-trial (Hypoxia and cell density imaging based boost for enhanced radiation therapy in head and neck affecting tumors)
In this trial we individualize radiation treatment based on biological properties visible through functional imaging comparable to molecular precision oncology. This is a fundamental change in radio oncological treatment approaches and will therefore have visionary character.

Preferred type of presentation:

15

Molecular and cellular mechanisms underlying the heterogeneity of disease manifestations in von Hippel–Lindau disease

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Oxygen is vital for aerobic organisms, which have evolved oxygen-sensing mechanisms to adapt to low-oxygen environments at the cellular level. The central effector in this pathway is hypoxia-inducible factor (HIF), whose α-subunits are regulated by the von Hippel–Lindau (VHL) tumor suppressor protein through proteasomal degradation. Functional inactivation of VHL results in stabilization of HIF-α, leading to constitutive activation of hypoxia-responsive genes and an increased risk of tumorigenesis. Germline mutations in the VHL cause von Hippel-Lindau syndrome, a disorder with a variable phenotype characterized by the development of various tumors, including renal cell carcinomas, hemangioblastomas, and pheochromocytomas. However, the molecular basis of this clinical variability remains poorly understood. Our current study in the SFB 1453 "NephGen"aims to decipher the molecular and cellular basis of this clinical heterogeneity by generating a comprehensive set of mutations by precision genome editing in an isogenic background of VHL wildtype-revertant kidney cancer cells.
This study builds on our previous project on nonstop mutations in VHL, which convert the stop into a sense codon, leading to translational readthrough until the next in-frame stop codon (Nat Cell Biol 2020, Nat Commun 2024). Nonstop mutations in VHL are enriched in renal cell carcinoma and result in proteins with short C-terminal extensions that trigger proteasomal degradation. Additionally, these mutations alter translation initiation and start site selection, favoring the expression of longer VHL isoforms (Sci Adv 2025).
Altogether, characterizing clinically relevant VHL mutations will advance our understanding of molecular and cellular mechanisms underlying the heterogeneity of VHL-associated disease manifestations.

Preferred type of presentation:

Poster Presentation only

16

Non-invasive genotyping and MRD monitoring by circulating tumor DNA in patients with solid tumors patients receiving targeted treatment within molecular tumor boards

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Introduction: Molecular tumor boards (MTB) stratify personalized targeted treatment for patients with rare and advanced cancers. Treatment response is assessed by CT/MRI, though limited by suboptimal sensitivity and specificity. Circulating tumor DNA (ctDNA) from blood plasma has emerged as a promising biomarker for noninvasive profiling of tumor mutational landscapes and disease monitoring. This study applied an ultra-sensitive next-generation sequencing (NGS) technology to evaluate ctDNA for tumor genotyping, early response prediction, and characterization of clonal heterogeneity in patients receiving targeted therapies within MTBs.
Methods: A custom-targeted NGS panel (ExTARGET) covering 266 genes across 540 kb, was applied to 167 plasma samples from 60 patients at distinct disease milestones. 24 healthy plasma samples were used to assess specificity. Digital droplet PCR (ddPCR) was used to assess concordance with NGS.
Results: In a pilot cohort of 21 patients, mutations were detected in 100% of pretreatment samples (median: 12 mutations/patient, range: 1-25). Target mutations guiding treatment initiation within MTBs, were identified in 80.9% (17/21 patients). Frequently mutated genes were *BRAF* (76%), *KRAS* (47%), *ROS1* (61%) and *TP53* (42%). NGS and ddPCR allele frequencies showed significant correlation (R^2=0.62, p<0.0001). Longitudinal tracking during therapy revealed that early ctDNA increases (4/4 cases) predicted disease progression at later timepoints. ctDNA dynamics reflected tumor burden and predicted progression in select patients.
Conclusion: We developed and implemented a NGS-based ctDNA profiling pipeline for patients with solid tumors within MTBs. Plasma genotyping identified targetable mutations in most cases. Monitoring ctDNA mirrors tumor burden and may enable early prediction of disease progression.

Preferred type of presentation:

17

Proteomic Profiling of iCCA: A Multicentric Study within the DKTK Network

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Intrahepatic cholangiocarcinoma (iCCA) is a rare and highly aggressive primary liver cancer associated with limited therapeutic options, late-stage diagnosis, and poor prognosis. Despite recent advances in genomic profiling, the biological complexity and clinical heterogeneity of iCCA remain poorly understood.
To address this, we are currently assembling a multicentric cohort of iCCA tumor samples through the German Cancer Consortium (DKTK) partner sites and the Clinical Communication Platform (CCP). Tumor samples will be processed using standardized and semi-automated protocols, followed by deep proteomic profiling via high-resolution liquid chromatography–mass spectrometry (LC-MS/MS). A central goal of this project is to integrate clinical parameters—including patient demographics, treatment histories, and outcomes—with proteomic data to identify molecular patterns associated with prognosis and therapeutic response.
The study builds on prior work from an independent American iCCA cohort, where proteomic analysis identified two major tumor subtypes: one enriched in extracellular matrix components, and another characterized by altered RNA processing and a higher risk of recurrence. Using a machine learning-based classification model, we could already validate these results in a local Freiburg iCCA cohort.
With the expanded DKTK cohort, we now aim to further validate and refine these molecular subtypes, discover novel protein-level biomarkers, and support the development of proteome-informed clinical strategies.

Preferred type of presentation:

Poster Presentation only

18

Decoding the spatial architecture of integrated electrophysiological and transcriptomic diversity in malignant brain tumors

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Glioma cells form synaptic connections with neurons, facilitating tumor progression and therapeutic resistance, yet the microenvironmental drivers of this synaptogenesis remain unclear. To investigate the mechanisms regulating neuron-glioma connectivity, we developed ElectroGenomics, an integrative spatial electrophysiology and transcriptomics approach combining high-density multielectrode array (HD-MEA) recordings, spatial transcriptomics, retrograde tracing, and graph-based network analysis. Applying this framework across human cortical slice cultures and murine glioma models, we found that tumor infiltration induces localized peritumoral hyperexcitability alongside inflammatory activation of microglia. In particular, inflammatory SPP1+/TREM2+ microglia, resembling damage-associated states observed in stroke and trauma, were found to drive BDNF-mediated synaptogenesis and facilitate neuron-tumor network formation through close spatial interactions with NPC/OPC-like tumor cells and sprouting neurons. Using optogenetic stimulation of cortical neurons in a patient-derived xenograft model, we confirmed that increased neuronal activity promotes the recruitment and activation of SPP1+ microglia specifically within the tumor-infiltrative regions. Pathway analysis further identified STAT3 signaling as a central driver of this inflammatory microglial phenotype. Pharmacological inhibition of STAT3 signaling or depletion of microglia significantly disrupted neuron-glioma connectivity and reduced neural circuit integration in human neocortical slice models. Complementary functional calcium imaging demonstrated that STAT3 inhibition led to decreased intratumoral signaling and diminished neuron-glioma synapse formation. Together, our study identifies inflammatory SPP1+/TREM2+ microglia as key regulators of neuron-glioma synapse formation and highlights the STAT3 pathway as a promising therapeutic target to disrupt glioma integration into brain circuitry.

Preferred type of presentation:

20

Spatial heterogeneity of CAR-T antigen expression in glioblastoma

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Background:
Chimeric antigen receptor T-cell (CAR-T) therapy has shown efficacy in multiple cancers but remains limited in glioblastoma due to tumor heterogeneity, immune evasion, and T-cell dysfunction. We analyzed spatial expression of CAR-T targets across tumor subtypes, between the tumor core and infiltration zone, and immune-reactive regions to identify therapeutic gaps.

Material and Methods:
We analyzed spatial transcriptomic data from 62 glioblastoma specimens using SPATA2 software, supported by advanced tools including scVI (data integration), Cell2Location (single-cell deconvolution), graph neural networks, gene set enrichment analysis (GSEA), and spatial annotation screening (SAS). CAR-T targets were selected based on existing clinical and preclinical studies.

Results: Spatial mapping revealed a gradient of NPC-like cells, enriched in the infiltration zone and resection margins—regions critical for residual disease. CAR-T targets such as ERBB2 and PTPRZ1 were highly expressed in AC-like and OPC-like niches but largely absent in NPC-rich areas, indicating a blind spot for current CAR-T therapies. Single-cell deconvolution linked NPC-like dominance to increased T-cell infiltration, while GSEA and clonal analysis suggested impaired T-cell function in these regions, indicating potential immune evasion.

Conclusion:
Our study exposes a fundamental limitation of current CAR-T designs: their inefficacy against NPC-like cells that dominate the infiltration zone and therefore the post-surgical glioblastoma ecosystem. By pinpointing spatial and phenotypic resistance hubs, we provide a preclinical rationale for adapting CAR-T strategies to address this gap and improve therapeutic coverage. Protein-level validation of key findings is currently ongoing to further substantiate the results.

Preferred type of presentation:

Poster Presentation only

21

From llama towards immunotherapy –A VHH binding domain enhances CLEC12A-CAR-NK cell therapy for treatment of AML

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Treatment of acute myeloid leukemia (AML) remains challenging due to its heterogeneity and lack of suitable target antigens. We recently discovered that treatment with hypomethylating agents, including 5-Azacytidine, induced the surface protein expression of CLEC12A (CLL-1) on leukemic blasts and leukemia-initiating cells in up to 92% of AML patients, but not on hematopoietic stem cells. This offers an attractive target for CAR-based immune cell therapy. Therefore, the NoviCARAZA joint funding project aims at developing a combination therapy of Azacytidine with CAR-engineered natural killer (NK) cells as off-the-shelf treatment option for hematological malignancies, combining targeted cytotoxicity and innate immune responses. To enhance anti-leukemic activity, we developed a CLEC12A-CAR-NK cell product featuring a novel VHH binding domain identified via llama immunization and yeast surface display. Combined with a 4-1BB-CD3ζ signaling domain and IL-15 armoring as CAR construct, these VHH-CAR-NK cells showed enhanced in vitro cytotoxicity compared to scFv-based CAR-NK cells and non-transduced NK cells. Cytotoxic efficiency was especially promising at low effector-to-target ratios. In an OCI-AML2 xenograft model, VHH-CAR-NK cells reduced tumor burden and increased survival more efficiently than the scFv-based counterpart, demonstrating their potential as effective AML-targeting cell therapy. Finally, advanced CRISPR/Cas9-based editing of the CLEC12A-CAR-NK cells led to further optimization of their high and promising anti-leukemic efficacy.

Preferred type of presentation:

22

The proteomic and immunopeptidomic landscape of non-small cell lung cancer

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Introduction: Non-small cell lung carcinoma (NSCLC), including adenocarcinoma (LUAD) and squamous cell carcinoma (LUSC), is the most common type of lung cancer, with distinct biological signatures. Accurate classification and molecular profiling are crucial for therapeutic guidance. In this study, we employed mass spectrometry (MS)-based proteomics to analyze a large patient cohort and investigate proteomic differences that may influence clinical behavior. High-coverage proteomics also offers insights into immune evasion mechanisms in NSCLC, while immunopeptidomics can identify tumor-associated antigens important for epitope-specific cancer immunotherapies.

Methods: Patient-derived samples were processed using an optimized, semi-automated SP3-beads workflow with a BRAVO pipetting robot. Extracted proteins were digested, and the resulting peptides analyzed by liquid-chromatography tandem-mass spectrometry (LC-MS/MS). Immunopeptidomics was performed using an automated HLA-I and HLA-II antigen enrichment workflow on the BRAVO platform. This adaptable affinity purification method processes various sample types, including fresh frozen tissue from biopsies.

Results: MS analysis of the cohort (LUAD n = 83, LUSC n = 70, tumor-adjacent n = 138) revealed over 10,000 unique proteins. Statistical analysis identified significant differences between LUAD and LUSC, with a potential distinction based on patient's gender. The immunopeptidomic workflow identified 44,417 unique HLA-I peptides and 27,626 unique HLA-II peptides, providing insights into antigen presentation. Additionally, de novo sequencing, a method that identifies peptide sequences without relying on a reference genome or database, was performed for both HLA-I and HLA-II peptides.

Outlook: The deep proteomic coverage offers insights into the molecular biology of LUAD and LUSC. Immunopeptidomics further promises the identification of tumor-specific antigens for each NSCLC subtype, facilitating the development of epitope-targeted immunotherapies. This work lays the foundation for personalized cancer immunotherapies tailored to individual tumor profiles.

Preferred type of presentation:

Poster Presentation only

23

Organoid-based response-prediction for targeted RAS+SHP2 and MEK/ERK+SHP2 inhibition in pancreatic cancer

Authors: Johana Carolina Noroña Alvarez[1]; Philipp Hafner[2]; Xun Chen[2]; Steffen Keller[2]; Asma Alrawashdeh[2]; Mara Schneider[2]; Hanna Scheffold[2]; Ting Chen[2]; Uwe Wittel[2]; Melanie Boerries[3]; Stephanie Mewes[2]; Kerstin Meyer[2]; Silke Hempel[2]; Geoffroy Andrieux[3]; Dietrich Ruess[3]

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Pancreatic ductal adenocarcinoma (PDAC) remains a leading cause of cancer-related mortality, underscoring the urgent need for novel effective therapeutic strategies. Although direct interference with mutant RAS signaling, the omnipresent oncogenic driver in PDAC, is currently revolutionizing targeted cancer therapy, resistance to RAS inhibitors emerges rapidly necessitating rational combination approaches. Furthermore, with a growing array of RAS inhibitor classes entering clinical development, predictive biomarkers to guide individualized treatment selection remain largely undefined.

To address this, we have established a continuously expanding biobank of to date >100 primary patient-derived organoid (PDO) cultures from both pretreated and treatment-naïve PDAC tumors. More than 40% of these models are viable after freeze–thaw cycles and amenable to comprehensive molecular and pharmacologic interrogation. Leveraging this resource, we are systematically assessing the differential efficacy of mutant-specific and mutant-agnostic RASon and RASoff inhibitors, alongside MEK/ERK inhibitors, all in combination with SHP2 blockade - a central node in resistance development in response to MAPK pathway interference.

Pharmacologic screening via high-throughput viability assays is accompanied by microscopy and molecular analyses focusing on RTK-RAS-MAPK signaling dynamics. Transcriptomic and genomic profiling (RNASeq, WES) of treatment-naïve and resistant organoids - matched to their parental tumor tissue - elucidate mechanisms underlying intrinsic and acquired resistance and allow exploration of correlations with therapy response. To validate in vitro findings, representative sensitive and resistant PDOs are orthotopically xeno-transplanted into immunocompromised mice for randomized treatment trials with RAS±SHP2 inhibitors. In vivo tumor dynamics are monitored via MRI, followed by histological and molecular assessment post-treatment.

This integrative platform aims to identify predictive genetic and transcriptomic biomarker signatures for response to RAS+SHP2 and MEK/ERK+SHP2 combination therapies in PDAC, with the ultimate goal of informing rational design of future clinical trials and guiding personalized therapy approaches.

Preferred type of presentation:

24

Characterisation of a Fluorescent Dye Library using a Dual-Labelled PSMA-617-based Tracer

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Introduction: In the field of dual-labelled tracers and fluorescence-guided surgery (FGS), ICG and IRDye800CW have been established as the most commonly used dyes. However, fluorescent dyes have continued to evolve. In order to investigate the suitability of such new dyes for potential clinical application, a library of dyes was conjugated to a PSMA-617-based tracer (DP). The aim was to identify a candidate with the most promising pharmacokinetic and optical properties to improve precise real-time feedback during surgery. **Materials and Methods:** Pharmacokinetic behaviour was assessed in BALB/c nu/nu mice bearing LNCaPPSMA+ xenografts by µPET/MRI at 1h and 2h after injection of 0.5 nmol (2.8 kDa - 3.2 kDa) of the 68Ga-labelled hybrid molecules. Fluorescence

was determined *ex vivo* as well as organ distribution 2 h p.i.. **Results:** *In vivo* studies revealed different pharmacokinetic profiles depending on the conjugated dye. Two candidates showed rapid clearance 1 h p.i. with high tumour uptake 2 h p.i.: DP-09 (9.14 %ID/g tumour, 26.27 %ID/g kidney) and DP-15 (7.67 %ID/g tumour, 7.68 %ID/g kidney). In contrast, DP-24 showed a slower clearance at 1 h p.i., but three times higher tumour accumulation compared to the others (22.94 %ID/g tumour, 61.97 %ID/g kidney). **Conclusion:** The three candidates identified were shown to have a promising pharmacokinetic profile after conjugation with the fluorescent dyes, making them suitable for further investigation. With improved photostability and higher quantum yields compared to ICG and IRDye800CW, the selected candidates may lead to an advantage in FGS.

Preferred type of presentation:

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Preclinical Evaluation of MT1-MMP-Targeting Bicyclic Peptides for Radiotheranostic Applications

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Membrane type 1 matrix metalloproteinase (MT1-MMP) is crucial in extracellular matrix degradation, which facilitates cancer progression and metastasis. Previous studies have shown the feasibility of using phage display-derived radiolabelled bicyclic peptides for positron emission tomography (PET) imaging. This study aimed to identify and characterize novel MT1-MMP-binding bicyclic peptides with an improved pharmacokinetic profile.

Four novel MT1-MMP-targeting bicyclic peptides (BCY1, BCY2, BCY3, and BCY4) were radiolabelled with either gallium-68 or lutetium-177. We assessed various physicochemical properties, including binding affinity, serum stability, and logD values. Cell binding and internalization were evaluated using MT1-MMP-expressing HT1080 cells. In vivo performance was analyzed in MT1-MMP+ tumor-bearing nude mice via μPET/MR imaging up to 2 hours post-injection (p.i.), followed by an organ distribution analysis (n = 3 for each peptide).
Results
All peptides exhibited nanomolar binding affinity (4.1 - 9.8 nM), with radiochemical purity exceeding 99 %. They demonstrated high serum stability, and their logD values ranged from - 3.6 to - 2.6. In vitro studies confirmed high specificity and minimal off-target binding (1 - 4 %). High tumour-to-background contrast was achieved within 30 minutes p.i. for BCY1, BCY2, and BCY4. Organ distribution studies revealed significant tumor uptake (12 - 25 % injected dose per gram, %ID/g) of all bicyclic peptides at 1-hour p.i., with BCY1 and BCY4 exhibiting the lowest off-target binding, aside from kidneys (5.8 %ID/g and 6.1 %ID/g, respectively).
Conclusion
BCY1 and BCY4 demonstrated optimal pharmacokinetic profiles characterized by high tumor uptake, specificity, and rapid blood clearance. Further translational research is essential to evaluate their full potential for radiotheranostic applications.

Preferred type of presentation:

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FreezeO –Advancing Personalized Cancer Therapy with Patient-Derived Organoids

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As three-dimensional in vitro models, patient-derived organoids (PDOs) closely mimic the original tumor's phenotypic and genotypic characteristics. We present FreezeO –the Biobank for Human (Tumor) Organoids, an initiative at the University Medical Center Freiburg dedicated to the isolation and culture of PDOs from tumor biopsies. FreezeO addresses a critical need for personalized therapy recommendations in advanced cancer.

The FreezeO core team primarily collects tumor samples from Molecular Tumor Board patients, generating three-dimensional PDO cultures and providing these valuable resources to researchers worldwide. Using standardized protocols, FreezeO enables rapid, personalized drug testing to identify patient-specific drug sensitivities. This approach holds significant potential for guiding clinical decision-making and improving patient outcomes.

To date, we have successfully biobanked PDOs from nine cancer types, including colorectal cancer (CRC), non-small cell lung cancer (NSCLC), and rare malignancies such as jejunal carcinoma. Notably, most PDOs originate from metastatic lesions, highlighting their potential benefits for late-stage cancer patients.

As an example, we present the case of FreezeO-21, a 46-year-old female patient with metastatic colorectal cancer (CRC) whose disease progressed over 5.5 years despite multiple treatments. A biopsy from a lung metastasis identified driver mutations in KRAS, SMAD4, and TP53, which remained stable across PDO passages, demonstrating the model's robustness. Targeted drug testing identified highly effective combination therapies, which were validated using Western blot analysis and RNA sequencing.

Preferred type of presentation:

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Patient-derived organoids (PDOs) as a companion tool to understand drug responses in the DKTK-funded SORATRAM IIT trial

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Class-III BRAF mutations are increasingly identified across all tumor entities. They render the kinase inactive and - contrary to what one might expect –can lead to strong upregulation of the MAPK pathway through dimerization and strong allosteric activation of other Raf kinases such as RAF1 (c-RAF). Due to the inactivity of the kinase, selective BRAF inhibitors such as Vemurafenib or Dabrafenib are ineffective. Hence, the pan-Raf inhibitor Sorafenib comes into play. To enhance its inhibitory effect on the MAPK pathway and to counteract paradoxical activation at low concentrations, Sorafenib is combined with the MEK inhibitor Trametinib. The DKTK-funded IIT SORATRAM is a basket trial investigating the efficacy of Sorafenib and Trametinib in cancer with kinase inactive BRAF mutations. For this study, biopsies from patients that were eligible for enrollment in the SORATRAM clinical trial were processed and patient-derived-organoids (PDOs) from their various tumor entities were established. Several SORATRAM drug tests were carried out to determine the responsiveness of the patients *in-vitro*. Through Western Blots and RNA-sequencing of PDOs, we gained a deeper insight into the mechanisms of the SORATRAM combination for each patient individually. Importantly, we could show that it is possible to confirm drug responses in PDOs while the patient is still receiving guideline therapies. We expect that this approach will guide therapeutic decisions in individual patients and will be invaluable to interpret the clinical data of SORATRAM.

Preferred type of presentation:

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Overcoming Sotorasib-resistance in KRASG12C-mutated patient-derived pancreatic cancer organoids

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Pancreatic ductal adenocarcinoma (PDAC) is a devastating cancer driven primarily by KRAS mutations. The KRASG12C mutation, while rare in PDAC, represents a targetable alteration with the inhibitors Sotorasib and Adagrasib approved for treatment of lung cancer. Early clinical trials show that while monotherapy with these inhibitors can provide initial clinical benefit in PDAC patients, all patients will eventually develop
progressive disease due to resistance mechanisms which are mostly unknown.To investigate these resistance mechanisms and to identify treatments to overcome resistance, we are inducing Sotorasib resistance in PDAC patient-derived organoids (PDOs) harboring the KRASG12C mutation. We aim to compare parental and resistant organoids to identify the transcriptional signatures driving resistance. This analysis will
include evaluating their differential responses to Sotorasib, Adagrasib, and and other emerging G12C inhibitors, as well as exploring strategic drug combinations that might overcome resistance. Initial characterization of the parental PDOs confirmed their sensitivity to both Sotorasib and Adagrasib, while KRASG12D-mutated and
KRASWT PDAC PDOs were resistant, demonstrating the mutation-specificity of these inhibitors. In addition, a drug combination screen in Sotorasib-sensitive, G12C-mutated PDOs identified combined KRASG12C and pan-ERBB inhibition via Afatinib as a promising, synergistic combination that could potentially delay or overcome resistance in this setting. Through molecular characterization and drug response profiling of
Sotorasib-resistant PDOs, we aim to identify additional therapeutic strategies for KRASG12C-mutated PDAC patients who develop resistance to targeted therapy.

Preferred type of presentation:

Poster Presentation only

30

Gliosarcoma: New Insights from Single Cell Sequencing and Spatial Transcriptomics

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Gliosarcoma is a rare and aggressive variant of glioblastoma, accounting for approximately 2% of all cases. Although it exhibits distinct histopathological and clinical features, it is currently managed using the same therapeutic strategies as glioblastoma and carries a similarly poor prognosis. In this study, we utilize single-cell RNA sequencing and spatial transcriptomics to investigate the molecular drivers underlying the formation of sarcomatous niches, which we hypothesize contribute to the unique clinical behavior of gliosarcoma. Preliminary findings reveal a distinct sarcomatous tumor cell phenotype absent in conventional glioblastoma, characterized by an enriched microenvironment of stromal and endothelial cells and elevated TGF-β signaling. By elucidating the cell–cell communication networks and signaling pathways specific to these niches, our aim is to uncover novel insights into gliosarcoma pathogenesis and identify potential therapeutic targets.

Preferred type of presentation:

Poster Presentation only

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Novel Harnessing of Innate and Adaptive Anti-Cancer Immunity: NLRP3-Inflammasome Activation Facilitates Immune Checkpoint Blockade in the Treatment of Mesenchymal Stage IV Colorectal Cancer

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Background: The role of the NLRP3 inflammasome in colorectal cancer (CRC) progression remains unclear (1). While some studies link high NLRP3 activity to cancer progression, others suggest protective roles, depending on the clinical context (2–4). Clinical trials mainly focus on inhibition of NLRP3 activity (5). Hence, we evaluated the effect of NLRP3 activation on metastatic CRC in vivo and in vitro.

Methods: In vitro experiments involved coculturing of peritoneal mouse immune cells with murine CRC organoids. Within, NLRP3 inflammasome activation was induced using EMT-244, a novel and potent NLRP3 activator. Therapeutic effects on the coculture were
quantified using viability assays. In vivo, we employed an orthotopic, organoid-driven mesenchymal stage IV CRC mouse model, which mimics an aggressive and treatment-resistant human CRC subtype (6). To evaluate the impact of EMT-244, immune histochemistry and fluorescence was performed.

Results: In vitro, we demonstrated an EMT-244-dependent reduction of CRC organoid viability during coculture ($p = 0.0498$). Importantly, this effect was not abundant when treating CRC organoids alone, highlighting the necessity of an immunological tumor microenvironment. In vivo experiments revealed that EMT-244, in combination with ICB,
significantly reduced liver and peritoneal metastatic burden compared to ICB monotherapy($p = 0.0114$ and $p = 0.0026$). Furthermore, liver metastases exhibited a significant decrease in Ki67 positivity ($p = 0.0157$) and an increase in TUNEL positivity ($p = 0.0388$) in the EMT-
244 + ICB group.

Conclusion: NLRP3 inflammasome activation by EMT-244 facilitates ICB in mesenchymal stage IV CRC, presenting a promising new immunological treatment approach for CRC metastases.

Preferred type of presentation:

Poster Presentation only

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Fatty Acid Synthase Inhibition in Colorectal Cancer Peritoneal Carcinomatosis

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Introduction
Peritoneal carcinomatosis (PC) in colorectal cancer (CRC) remains a major therapeutic challenge. We previously identified elevated fatty acid metabolism in PC-derived cancer stem cells (CSCs) compared to liver metastases (LM) and primary tumors (PT) from a murine stage IV CRC model. Western blot analyses confirmed significantly higher expression of fatty acid synthase (FASN) and acetyl-CoA carboxylase (ACC) in epithelial cells from PC. These findings suggest a metabolic vulnerability that may be targeted therapeutically.

Methods
We evaluated the therapeutic potential of FASN inhibition in a CRC PC mouse model treated with the FASN inhibitor Fasnall. The peritoneal carcinomatosis index (PCI), liver, and primary tumor burden were assessed. Organoid re-seeding assays from PC, LM, and PT were performed to analyze CSC function and proliferation following Fasnall treatment. Apoptosis was quantified using Annexin V FACS analysis.

Results
Fasnall treatment significantly reduced PCI in vivo, without affecting liver or primary tumor burden. In organoid re-seeding assays, Fasnall treatment markedly decreased organoid formation, particularly in PC organoids, indicating impaired CSC function. Fasnall treatment resulted in increased apoptosis and reduced proliferation in organoid cultures.

Conclusion
Our results indicate that fatty acid metabolism represents a promising therapeutic target in CRC PC. FASN inhibition by Fasnall impairs PC growth and may disrupt CSC function, highlighting its potential for innovative treatment strategies in this difficult-to-treat disease subset.

Preferred type of presentation:

Poster Presentation only

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Metabolic surgery reduces CRC disease progression mediated by circulating bile acid diversion

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Obesity is a global epidemic characterized by energy disequilibrium, metabolic disorders, and fat mass development that greatly affect the health status of individuals. There is evidence that the intake of a high-fat diet and overweight are associated with the incidence of colorectal cancer (CRC). Metabolic surgery has been associated with improvements in obesity-related comorbidities and a reduction in the overall cancer risk. However, the underlying mechanism by which metabolic surgery reduces the risk of CRC remains unknown. To understand the anti-tumoral mechanism of bariatric surgery, we analyzed the development of CRC after Roux-en-Y gastric bypass surgery (RYGB) in a RYGB-CRC mouse model. Here, we showed that RYGB surgery substantially reduced primary tumorigenesis and prevented metastasis. This protective effect was mediated by bile acid (BA) exclusion from the proximal small intestine, leading to BA diversion in the preceding parts of the gastrointestinal tract and in circulation. The diverted BA profile in RYGB mice showed anti-tumoral and anti-metastatic effects that were verified by BA exclusion of the proximal small bowel without the systemic metabolic installations of RYGB surgery by a cholezysto-intestinal shunt (CIS) surgery. RYGB surgery thus leads to reduced primary BAs and elevated secondary BAs in circulation. In a translational study involving patients with CRC with metachronous liver metastases (CRLM), we confirmed that reduced primary bile acid concentrations in the serum were associated with prolonged time to metastasis, underscoring the critical role of bile acids in CRC progression and metastatic development.

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Platform Intro: FREEZE Biobank

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Michael Quante: Prevention and Early Detection of Esophageal Adenocarcinoma with Fluorescence Molecular Endoscopy

Invited Speaker

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Evelyn Ullrich: From llama towards immunotherapy –A VHH binding domain enhances CLEC12A-CAR-NK cell therapy for treatment of AML

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Platform Intro: FREpi : a curated epigenetic inhibitor screening library for target discovery and validation

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Wrap Up by Melanie Börries and Robert Zeiser