MAX DELBRÜCK CENTER

DFG

LMU LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN

BERLIN BRAIN TUMOR MEETING 2024

INVITED SPEAKERS

Britta Engelhardt
Theodor-Kocher-Institut (TKI), Switzerland

Yi Fan
University of Pennsylvania Perelman School of Medicine, USA

Mariella Filbin
Dana-Farber/ Boston Children's Cancer & Blood Disorder Center Harvard Medical School, USA

Gaetano Gargiulo
Max-Delbrück-Center for Molecular Medicine

Kiavash Movahedi
Vrije Universiteit Brussel, Belgium

Marco Prinz
Neurozentrum Albert-Ludwigs-Universität Freiburg, Germany

Saverio Tardito
The Beatson Institute for Cancer Research, UK

Varun Venkataramani
University of Heidelberg, Germany

SELECTED SPEAKERS

Haris Babačić
Karolinska Institute, Sweden

Krishna Bhat
Mayo Clinic, USA

Srijita Banerjee
Uppsala University, Sweden

Lola Boutin
Karolinska Institute, Sweden

Guillaume Bourmeau
Institut Curie, France

Roland Friedel
Mount Sinai, USA

Nils R. Hebach
University of Heidelberg, Germany

Niels Olshausen
University of Heidelberg, Germany

Aylin Möckl
University of Tübingen, Germany

Scientific Committee
Gaetano Gargiulo, MDC
Rainer Glass, LMU
Charlotte Flüh, Göttingen
Christoph Harms, Charité
Franz Josef Müller, MPI Berlin

MAY 23(14:00)-24 (16:00), 2024

MAX-DELBRÜCK-CENTER,

ROBERT-RÖSSLE-STR. 10, 13125 BERLIN

HELMHOLTZ

UKSH UNIVERSITÄTSKLINIKUM Schleswig-Holstein

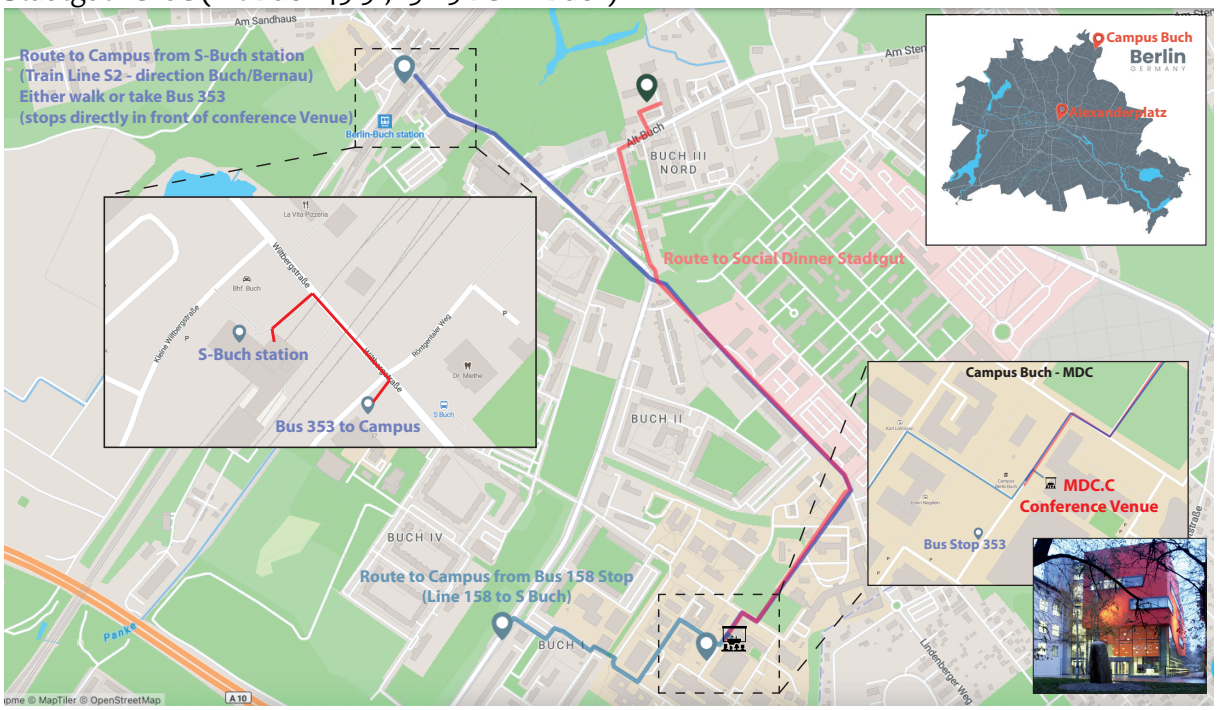
CAU Christian-Albrechts-Universität zu Kiel

MAX-PLANCK-INSTITUT FÜR MOLEKULARE GENETIK

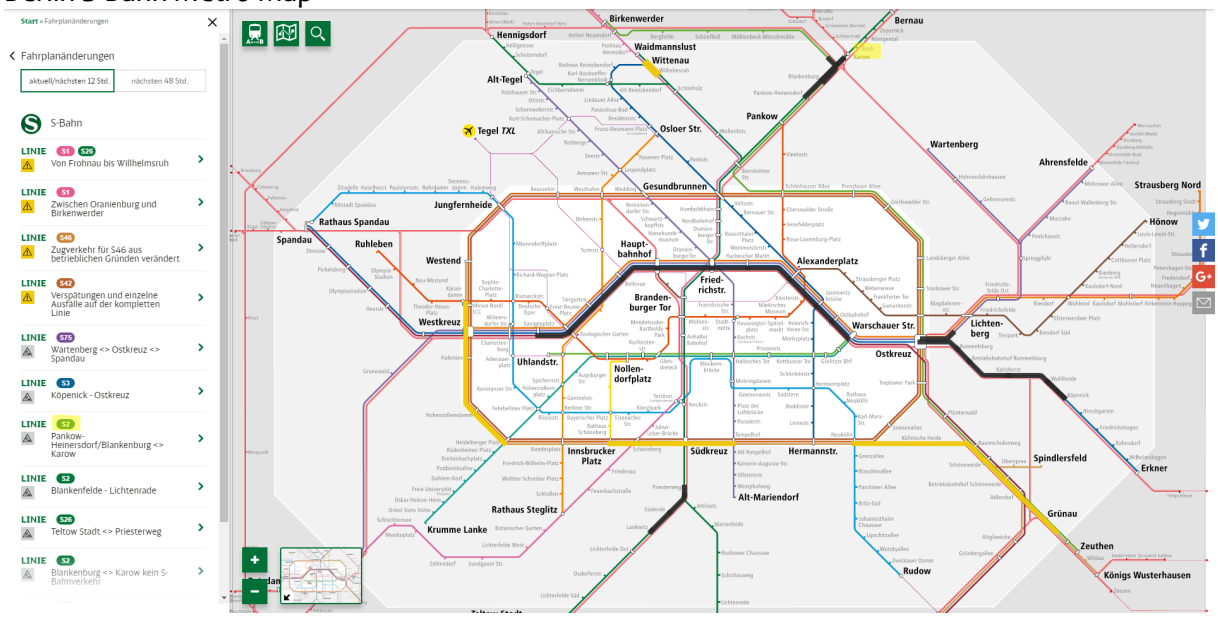
CHARITÉ UNIVERSITÄTSMEDIZIN BERLIN

Campus Buch

Stadtgut venue (Alt-Buch 45-51, 13125 Berlin Buch)



Berlin S-Bahn Metro Map



Thursday, May 23, 2024

14:00 – 14:05	**Welcome Address: Gaetano Gargiulo and Rainer Glass**
14:05 - 15:25	**Session I: Pediatric brain tumors** **Chair: Gaetano Gargiulo**
14:05 – 14:45	Plenary I, Speaker: **Mariella Filbin**, Dana-Farber/Boston Children's Cancer and Blood Disorder Center Developmental pathways and plasticity of pediatric high-grade glioma
14:45 – 15:05	**Srijita Banerjee**, Matched primary-relapse pedHGG patient-derived xenograft models to identify therapy resistance profiles
15:05 - 15:25	**Lola Boutin**, Gamma Delta T cell recognition and activation potential in Medulloblastoma
15:25 – 16:10	**Poster Session and Coffee Break**
16:10 – 17:40	**Session II: Vascular biology in the CNS and brain tumors** **Chair: Rainer Glass**
16:10 – 16:50	Plenary II, Speaker: **Britta Engelhardt**, Theodor Kocher Institut The role of the brain barriers in maintaining CNS homeostasis and immune privilege
16:50 - 17:30	Plenary III, Speaker: **Yi Fan**, University of Pennsylvania Vascular regulation of glioma immunity
17:30 – 18:15	**Coffee Break and Poster Session**
18:15 – 19:35	**Session III: Brain tumor cell and molecular biology** **Chair: Charlotte Flüh**
18:15 – 18:55	Plenary IV, Speaker: **Gaetano Gargiulo** Cell states and fate transitions in glioblastoma revealed by synthetic genetic tracing
18:55 – 19:15	**GuillaumeBourmeau**: Tracking glioblastoma cell plasticity identifies GB-Hybrid, a therapy resistant cell state dependent on nuclear import
19:15 – 19:35	**Roland Friedel:** Regulation of membrane tension by Plexin receptors facilitates diffuse invasion of GBM
20:00 – 23:00	**Reception at the Stadtgut Buch Barn**
20:15 – 20:45	Short pitches from poster presenters, travel grant holders and industry partners.
21:00 – 21:30	Short talks on selected topics

Friday, May 24, 2024

9:00 - 10:20	**Session IV Brain tumor microenvironment and metabolism** **Chair: Christoph Harms**
9:00 - 9:40	Plenary V Speaker: **Saverio Tardito**, Cancer Research UK Scotland Institute Steroids-dependent metabolic rewiring reveals novel therapeutic and imaging approaches for glioblastoma
9:40 – 10:00	**Haris Babačić**, Longitudinal blood plasma proteomics stratifies glioblastoma patients in two groups that differ in overall survival
10:00 - 10:20	**Krishna Bhat**, Single Cell Genomics Identifies Anti-Glioma Phagocytic Immunomodulators
10:20 – 11:00	**Poster Session and Coffee Break**
11:00 – 12:20	**Session V: Macrophage biology in the CNS and brain tumors** **Chair: Franz Josef Müller**
11:00 – 11:40	Plenary VI Speaker: **Marco Prinz**, Freiburg University The myeloid side of the brain
11:40 – 12:20	Plenary VII Speaker: **Kiavash Movahedi**, Vrije Universiteit Brussel Single-cell mapping and modulation of brain immune ecosystems
12:20 – 13:30	**Lunch and Poster Session**
13:30 – 14:10	**Session VI Brain Metastasis** **Chair: Michela Serresi, MDC**
13:30 – 13:50	**Aylin Möckl**, Dissecting the role of CSF2Rb-STAT5 signaling in tumor-associated inflammation in brain metastasis
13:50 – 14:10	**Nils R. Hebach**, The Aging Microenvironment exacerbates Leptomeningeal Metastasis
14:10 – 14:40	**Coffee Break and Poster Session**
14:40 – 15:40	**Session VII: Cancer Neuroscience** **Chair: Pilar Sanchez-Bailon, MDC**
14:40 – 15:00	**Niels Olshausen**, Spontaneous Cancer Cell Intrinsic Calcium Oscillations drive Brain Metastasis Progression
15:00 – 15:40	Plenary VIII Speaker: **Varun Venkataramani**, Heidelberg University Brain-Wide Neural Circuits Of Glioblastoma Drive Tumor Progression
15:45 – 16:00	Farewell address: G. Gargiulo, R. Glass
16:00	**End of BTM2024**

Flash talks at Extended event (Stadtgut Buch)

First Name	Last Name	Institute	Country
Tanja	Eisemann	Sanford Burnham Prebys	USA
Katarzyna	Leszczynska	Nencki Institute of Experimental Biology	Poland
Giulia	Marotta	European Institute of Oncology (IEO)	Italy
Dominic	Menger	M3 Research Center Tübingen	Germany
Katja	Nadler	Fraunhofer ISC (TLC-RT)	Germany
Verena	Panitz	Deutsches Krebsforschungszentrum Heidelberg	Germany
Patryk	Rurka	University of Silesia Katowice	Poland
Anirudh	Sattiraju	Icahn School of Medicine at Mount Sinai	USA
Lois	Yardy	StandardBio	Germany
Irene	Yujnovsky	Nanostring	Germany

GENERAL INFORMATION
May 23, 2024, 2:00pm - May 24, 2024, 4:00pm
Venue MDC C83
Robert-Rössle-Straße 10
13125 Berlin
Germany

Registration
The registration desk will be located at the meeting venue (MDC C83, ground floor) and will be staffed during all breaks.

Registration fee includes

- admission to the lecture hall and the poster/exhibition area
- free coffee breaks
- conference material and name tag

On-site registration is possible, but payment should be made via the website. If you have any questions, please feel free to ask at the registration desk.

Wireless Internet at the Meeting Venue Network:
MDC_OPEN

Talks
All talks will take place in the Axon I and II conference rooms.
Duration of Plenary Lectures: 30 min. (talk) + 10 min. (disc)
Duration of Oral Presentations: 15 min. (talk) + 5 min. (disc)

Invited speakers and short oral presenters are requested to deliver their slides during the break prior to their session. If speakers are using their own computer, please check the connection first.

Photography or video/audio recording of any material or persons requires permission from the author(s) and the workshop organizers. Posting scientific material presented at this meeting on social media platforms is restricted and requires explicit permission from the presenting author(s).

Poster Sessions
Poster sessions will feature all posters simultaneously at MDC C83, ground floor. Each attendee will have a unique poster stand, with a number associated with their abstract. If you are presenting a poster, please feel free to attend your stand or others at your convenience.
The size of the posters is 1 - 1.20 m height and 1 m width. Larger formats cannot be accommodated on the boards.

Poster Awards
The three most voted posters will be announced in the final address on Friday, 24th. Each attendee will have a unique QR code for voting on one poster, located on their name tags. Organizers can vote for multiple posters.
The deadline for casting the votes is Friday, May 24 at noon (15:00 h).

Coffee Breaks and Lunch
Coffee breaks will be served in parallel with the poster session in the Foyer of MDC C83, ground floor.

Lunch options are available at the MDC canteen (A14) and food trucks on-site. Additional options are available at Café Max (A8) or the cafeteria in H31.1.
Please note that there may be significant queues.

Extended Event Including Flash Talks and Social Dinner
Flash talks will take place at the Stadtgut venue (Alt-Buch 45-51, 13125 Berlin Buch – see map) in parallel with the social dinner. Each talk will last a maximum of 3 minutes.

Please note, this event is restricted to invited speakers and paid participants (extended event fee, or late-bird registration rate). The event is fully booked, but please check in with the registration desk if you wish to purchase a ticket. In case of cancellations, tickets may become available, but this is not guaranteed.

Exhibition
Commercial booths are located in the poster area and are open during the meeting hours.

Insurance
The organizers do not take responsibility for individual medical, travel or personal insurance. Participants are advised to carry out their own insurance policy.

Language
The official meeting language is English.

Credit points for medical doctors practicing in Germany
The following message concerns medical doctors practicing in Germany only:
Alle Brain Tumor Meetings sind bisher von der Berliner Ärztekammer als Fortbildungsveranstaltung für Mediziner anerkannt worden, so dass Teilnehmer an der Tagung Fortbildungspunkte erhalten. Auch für das Brain Tumor Meeting 2022 wurde die Anerkennung wieder beantragt. 2017 hatte die Ärztekammer insgesamt 10 Punkte (5 Punkte für Donnerstag und 5 Punkte für Freitag) vergeben. Für die Meldung der Teilnehmer an die Ärztekammer liegen Teilnehmerlisten aus, in die sich die an Fortbildungspunkten interessierten Teilnehmer eintragen müssen. Dafür wird ein Barcode-Etikett benötigt. Bitte bringen Sie diese zum Einkleben mit. Für jeden Veranstaltungstag wird eine seperate Liste ausliegen. Zusätzlich werden Teilnahmezertifikate ausgestellt.

Early career researcher travel grants
Travel grants were granted to early career researchers qualified for participation in the Brain Tumor Meeting 2024. Selected awardees:

Helena Margarida Rodrigues de Figueiredo, University of Minho, Braga Portugal
Deborah Gargano, University of Molise
Patryk Rurka, University of Silesia in Katowice
Miriam Russo, University of Molise
Tina Kolenc-Milavec, National institute of Biology, Ljubljana, Slovenia

BTM2024 Organizing Committee:
- Gaetano Gargiulo, Max Delbrück Center, Berlin
- Rainer Glass, Ludwig-Maximilians-Universität, Munich
- Charlotte Flüh, University Hospital of Göttingen
- Christoph Harms, Charité-Universitätsmedizin Berlin
- Franz Josef Müller, Max Planck Institute for Molecular Genetics, Berlin

Organisational Inquiries:
MDC Events Team
Max Delbrück Center for Molecular Medicine in the Helmholtz Association (MDC)
Email: events@mdc-berlin.de

Speaker Abstracts

Developmental pathways and plasticity of pediatric high-grade glioma

Mariella Filbin[1,*]

[1]Dana-Farber/Boston Children's Cancer and Blood Disorder Center
*Presenting author

Pediatric High-grade gliomas are driven by unique, location-specific histone mutations and are uniformly lethal. Here, I will discuss how we elucidate the cell state composition and unique architecture of these glioma types, and discuss their similarities with normal brain development.

Supported by SciSerTec
Web: http://www.scisertec.de

The role of the brain barriers in maintaining CNS homeostasis and immune privilege

Britta Engelhardt[1],

[1]Theodor Kocher Institute, University of Bern, Bern, Switzerland

Central nervous system (CNS) neurons coordinate all our body functions by high speed electrical and chemical communication. To achieve this complex task they need a strictly regulated homeostatic environment, which does not tolerate uncontrolled entry of blood components including immune cells. The CNS has thus developed a unique relationship with the immune system referred to as CNS immune privilege. The brain barriers are the endothelial blood-brain barrier (BBB) at the level of CNS parenchymal micro vessels, the epithelial blood-cerebrospinal fluid barrier (BCSFB) of the choroid plexuses (ChP) in the brain ventricles, the arachnoid barrier (AB) formed by meningeal fibroblasts on the CNS surface and the evolutionary older brain barrier, the glia limitans formed by astrocytes enclosing the entire CNS parenchyma. The brain barriers establish a CNS zonation that can be compared to the architecture of a medieval castle that is surrounded by two walls bordering a castle moat. In this view the CNS parenchyma hosting the neurons is the castle and the cerebrospinal fluid (CSF) filled spaces resemble the castle moat which is bordered by outer and inner brain barriers and allows for immune surveillance. Under neuroinflammatory conditions immune cells invade the CNS parenchyma leading to breakdown of CNS homeostasis and brain barrier functions. Understanding the orchestrated function of the different brain barriers is of fundamental importance to understand how these interfaces shape CNS immunity and neuronal function and thus ultimately CNS health and disease.

Supported by SciSerTec

Web: http://www.scisertec.de

Vascular regulation of glioma immunity

Yi Fan[1,*]

[1]University of Pennsylvania
*Presenting author

Cancer immunity is subject to spatiotemporal regulation by leukocyte interaction with the tumor microenvironment. Growing evidence suggests an emerging role for the vasculature in tumor immune evasion and immunotherapy resistance. As such, glioma is characterized by prominent abnormal vascularity, and exhibits high resistance to T cell-based immunotherapy. Beyond the conventional functions of the tumor vasculature, such as providing oxygen and nutrients to support tumor progression, we propose multiplex mechanisms for vascular regulation of tumor immunity: The tumor vascular endothelial cells (ECs) locoregionally interact with and educate circulation-derived myeloid cells including macrophages and myeloid-derived suppressor cells (MDSCs), leading to formation of an immunosuppressive vascular niche; ECs release excessive immunosuppressant metabolites that induce T cell exclusion and inactivation; topologically and biochemically abnormal vascularity disrupts lymphocyte adhesion and forms a pathophysiological barrier that hampers T cell infiltration. We postulate that genetic and metabolic reprogramming of tumor ECs may rewire the immunosuppressive vascular microenvironment to activate host anti-tumor immunity and to overcome immunotherapy resistance, serving as a next-generation strategy for cancer treatment.

Cell states and fate transitions in glioblastoma revealed by synthetic genetic tracing

Gaetano Gargiulo[1,*]

[1]Max-Delbrück-Center for Molecular Medicine (MDC), Robert-Rössle-Str. 10, 13092 Berlin, Germany
*Presenting author

Glioblastoma (GBM) is a lethal brain tumor with limited therapeutic options. The efficacy and bioavailability of approved therapeutics, as well as the discovery of new ones, are hindered by the blood-brain barrier (BBB) and the dynamic changes in tumor cell states. In my presentation, I will discuss our innovative approach to studying cell identities, states, and fate transitions in glioblastoma using synthetic genetic tracing. Our previous research has demonstrated that GBM cells adapt in response to standard therapies and interactions with innate immune cells. Specifically, we found that myeloid cells drive a mesenchymal state in GBM cells, which promotes acquired resistance to therapeutics.
I will provide proof-of-concept evidence showing how this approach can be translated into a phenotypic drug discovery (PDD) platform. This platform replicates the pathophysiologically relevant interaction between GBM and innate immune cells in vitro, leading to phenotypic transitions and a shift in drug sensitivity within a multicellular 3D model. I will present preliminary data obtained using this platform, demonstrating how the categorization of drug treatments based on tumor cell viability, adaptive cell state changes, and the survival of non-transformed innate immune cells has led to the prioritization of small molecules and biologicals capable of modulating these parameters. Finally, I will show an example of how our PDD has uncovered previously unrecognized therapeutic responses for a clinically relevant drug.
Our data highlight the potential of synthetic genetic tracing to advance the discovery of therapeutics for the combinatorial targeting of cell states and phenotype switching, and support the race towards overcoming therapy resistance and improving treatment outcomes for GBM patients.

Steroids-dependent metabolic rewiring reveals novel therapeutic and imaging approaches for glioblastoma

Saverio Tardito[1, 2,*]

[1]Cancer Research UK Scotland Institute, Garscube Estate, Switchback Road, Glasgow, G61 1BD, UK
[2]University of Glasgow
*Presenting author

Dexamethasone, a steroid anti-inflammatory drug, is invariably used to manage brain tumour-associated oedema but its direct effects on brain tumour metabolism are largely unknown. An untargeted metabolomics screen in a panel of naïve glioblastoma cells treated with dexamethasone revealed the consistent accumulation of N1-methylnicotinamide, an observation validated in surgical samples from glioblastoma patients. Dexamethasone-dependent activation of the glucocorticoid receptor promoted the transcription of Nicotinamide N-methyltransferase (NNMT), whose activity assessed by stable isotope-assisted metabolomic analysis, is selectively enhanced in orthotopic glioblastoma tumours compared to contralateral brain tissue. The nicotinamide methylation consumes one carbon units from methionine becoming detrimental for glioblastoma tumour proliferation when dexamethasone treatment is associated with a methionine-restricted diet. Finally, we show that the NNMT-dependent trapping of nicotinamide can visualize glioblastoma tumours by radiometabolic imaging. Overall, steroids rewire methionine and nicotinamide tumour metabolism that can be clinically exploited for imaging and therapy of glioblastoma patients.

The myeloid side of the brain

Marco Prinz[1,*]

[1]Freiburg University
*Presenting author

The innate immune compartment of the human central nervous system (CNS) is highly diverse and includes several immune-cell populations such as macrophages that are frequent in the brain parenchyma (microglia) and less numerous at the brain interfaces as CNS-associated macrophages (CAMs). Due to their scantiness and particular location, little is known about the presence of temporally and spatially restricted CAM subclasses during development, health and perturbation. Here we combined single-cell RNA sequencing, time-of-flight mass cytometry and single-cell spatial transcriptomics with fate mapping and advanced immunohistochemistry to comprehensively characterize the immune system at human CNS interfaces with over 356,000 analyzed transcriptomes from 102 individuals. We also provide a comprehensive analysis of resident and engrafted myeloid cells in the brains of 15 individuals with peripheral blood stem cell transplantation, revealing compartment-specific engraftment rates across different CNS interfaces. Integrated multiomic and high-resolution spatial transcriptome analysis of anatomically dissected glioblastoma samples shows regionally distinct myeloid cell-type distributions driven by hypoxia. Notably, the glioblastoma-associated hypoxia response was distinct from the physiological hypoxia response in fetal microglia and CAMs. Our results highlight myeloid diversity at the interfaces of the human CNS with the periphery and provide insights into the complexities of the human brain's immune system.

Single-cell mapping and modulation of brain immune ecosystems

Kiavash Movahedi[1, *]

[1]Vrije Universiteit Brussel
*Presenting author

Brain-immune interactions and immunosurveillance are essential for maintaining healthy brain homeostasis. Key hubs for neuro-immune regulation are found at the brain's border tissues, which form the interface with the periphery. Border sites, such as the meninges, have emerged as the main immune gateways to the brain, harbor a rich immune landscape and are likely involved in immune responses against primary brain tumors. While single-cell transcriptomics has helped to reveal the immune heterogeneity within these tissues, spatial profiling remains challenging and has not been thoroughly addressed. We have now developed a pipeline for single-cell proteomic mapping of the intricate immune ecosystems that are found in the brain's border regions, offering new opportunities for understanding neuro-immune regulation in the brain. Additionally, I will discuss the central role played by macrophages in these ecosystems and how this is related to brain tumor progression. Macrophage and microglia engineering and replacement offer great therapeutic opportunities but has remained challenging. I will present new insights into the dynamics of brain macrophage replacement, both for mouse and human cells.

Brain-Wide Neural Circuits Of Glioblastoma Drive Tumor Progression

Varun Venkataramani[1,*]

[1]Heidelberg University
*Presenting author

Glioblastomas are highly invasive, yet incurable brain tumors characterized by their notorious therapeutic resistance. Cancer cells are known to organize and communicate within functional networks, akin to those observed in biological and sociological systems, which are crucial for their growth and resilience. More recently, neuron-to-glioma synapses have been shown to promote glioblastoma progression. However, characterizing tumor-connected neurons has been challenging due to a lack of suitable technologies. Recently, we adapted retrograde tracing using rabies viruses to investigate and manipulate neuron-tumor networks, revealing that glioblastomas rapidly integrate into neural circuits across the brain and engage in widespread functional communication. Acetylcholinergic neurons, in particular, drive glioblastoma invasion. Our findings uncovered patient-specific and tumor cell state-dependent differences in synaptogenic gene expression associated with neuron-tumor connectivity and subsequent invasiveness. Importantly, radiotherapy was found to enhance neuron-tumor connectivity by increasing neuronal activity. Conversely, simultaneous neuronal activity inhibition and radiotherapy showed improved therapeutic effects, indicating that neuron-to-glioma synapses contribute to therapeutic resistance. Finally, rabies-mediated genetic ablation of tumor-connected neurons halted glioblastoma progression, presenting a viral strategy to combat glioblastoma. Together, this study provides a comprehensive framework for characterizing neuron-tumor networks and targeting glioblastomas, with potential translational implications for other tumor types and ongoing clinical trials based on these discoveries.

Abstracts

Abstract No. 01

Analysis of temporal and regional expression changes and co-staining patterns of metabolic and stemness markers during glioblastoma progression

Lea Gilles[1,*], *Carolin Kubelt*[1], *Dana Hellmold*[1], *Tjorven Blumenbecker*[1], *Eva Peschke*[2], *Olga Will*[2], *Hajrullah Ahmeti*[1], *Jan-Bernd Hövener*[2], *Olav Jansen*[3], *Ralph Lucius*[4], *Michael Synowitz*[1], and *Janka Held-Feindt*[1]

[1]Department of Neurosurgery, University Medical Center Schleswig-Holstein UKSH, Campus Kiel
[2]Molecular Imaging North Competence Center, Department of Radiology and Neuroradiology, University Medical Center Schleswig-Holstein, Campus Kiel
[3]Department of Radiology and Neuroradiology, University Medical Center Schleswig-Holstein, Campus Kiel
[4]Institute of Anatomy, Kiel University
*Presenting author

Metabolic alterations facilitate growth and therapeutic resistance of glioblastoma (GBM). Likewise, a subpopulation of GBM cells, so-called glioma stem-like cells (GSCs), contribute to these aggressive properties. Relating to metabolism, GSCs seem to be less glycolytic than the tumor bulk, however, they are able to switch between oxidative phosphorylation and aerobic glycolysis. Since further knowledge of the metabolism of GSCs could be of clinical use, we focused on the connection between metabolic alterations and stemness by analyzing temporal and regional expression changes and co-staining patterns of metabolic [pyruvate kinase muscle isozyme 1/2 (PKM1/2), glucose transporter 1 (GLUT1), monocarboxylate transporter 1/4 (MCT1/4)] and stemness markers [Nestin, Krüppel-like factor 4 (KLF4)] during GBM progression in a rodent model and patient-derived samples. Rat tumor biopsies revealed a temporally increasing expression of GLUT1, higher expression of MCT1/4, Nestin, and KLF4, and lower expression of PKM1 compared to contralateral hemisphere. In patient-derived tumors a higher expression of PKM2 and Nestin in tumor center vs. edge was observed. Whereas rare co-staining of GLUT1/Nestin was found in tumor biopsies, PKM1/2, and MCT1/4 revealed a more distinct co-staining with Nestin in rats and humans. In human and rat tumors KLF4 was mainly co-stained with GLUT1, MCT1, and PKM1/2. All of the metabolic markers yielded individual co-staining patterns among themselves. Co-staining mainly occurred later in terms of tumor progression and more pronounced in tumor centers. Finally, positive correlations were found amongst markers that showed co-staining. Our results highlight a link between metabolic alterations and stemness in GBM progression, with complex distinctions depending on the respective markers, time points, and regions studied.

Abstract No. 02

GD3, a promising therapeutic target against glioblastoma stem cells

Nora Essakhi[1,*], *Victoria Hein*[2], *Nathalie Baeza-Kallee*[3], *Raphaël Bergès*[4], *Aurélie Soubéran*[2], *Carole Colin*[2], *Aurélie Tchoghandjian*[2], *Dominique Figarella-Branger*[2], and *Emeline Tabouret*[2]

[1]Institute of neurophysiopathology (INP)
[2]Institute of Neurophysiopathology (INP)
[3]Institute of neurophysiopathologie
[4]Inst Neurophysiopathol
*Presenting author

Glioblastoma is the most common and aggressive primary brain tumor in adults. There is no curative treatment, and therapeutic resistance is partly related to the presence of cancer stem cells and immunosuppressive microenvironment. Identifying relevant targets for these cells and developing dedicated immunotherapy is therefore a therapeutic challenge. Our team has previously demonstrated that gangliosides can be linked to stem-like properties of glioblastoma cells. Our objective was to demonstrate that GD3 was a relevant epitope for glioblastoma stem cells (GSC).
We sorted GD3 positive cells from fresh patient samples and derived GD3+ cell lines. GD3 synthase (ST8SIA1) was downregulated by using shRNA in various glioblastoma cells lines. Furthermore, tumorigenicity of cell models was assessed in nude mice. Then, bulk RNAseq screening was performed to decipher the impact of shST8SIA1.
Firstly, we showed that GD3 was expressed by 20% of cells from patient bulk tumors but not by normal brain. Then, we showed that our patient-derived GSC lines strongly expressed GD3 and, importantly, this GD3 population decreased significantly after cell differentiation. GD3-positive cells sorted from patient samples had stem properties: they were plastic, generated SOX2+ and NESTIN+ spheres, had clonogenicity properties and were tumorigenic after orthotopic graft. Then, using shST8SIA1 in GSC lines, we showed that silencing of ST8SIA1/GD3 was associated with a decrease in sphere size, self-renewal and migratory capacities and increased mouse survival. Furthermore, reduced ST8SIA1/GD3 expression increased sensitivity to temozolomide and radiotherapy in vitro.
Finally, data from pan-transcriptomic analysis of shST8SIA1 GSC lines showed that silencing ST8SIA1/GD3 decreased oncogenic pathways and more specifically the expression of ADAMTS1 and IL33, that we confirmed by RTqPCR.
Taken together, our results strongly suggest that GD3 and ST8SIA1 are essential for glioblastoma stem cell properties. GD3 appears to be a promising GBM stem cell epitope, opening promising therapeutic development against glioblastoma.

Abstract No. 03

A novel role of exostosin glycosyltransferase 2 (EXT2) in glioblastoma cell metabolism, radiosensitivity and ferroptosis

Rocio Matesanz-Sanchez[1], *Mirko Peitzsch*[2], *Michael Seifert*[3], *Nils Cordes*[1, 4], and *Anne Vehlow*[1,*]

[1]OncoRay – National Center for Radiation Research in Oncology
[2]Institute of Clinical Chemistry and Laboratory Medicine
[3]Institute of Medical Informatics and Biometry (IMB)
[4]Institute of Radiooncology – OncoRay
*Presenting author

Inherent resistance of glioblastoma (GBM) cells to therapy contributes significantly to the dismal prognosis of glioblastoma patients. Besides genetic and epigenetic alterations, metabolic reprogramming appears fundamental in this aspect. Recent studies suggest a connection between therapy resistance and ferroptosis, an emerging cell death mechanism associated with lipid peroxidation. Nevertheless, the causal circuits are only partially understood. Here, we identify novel regulators of therapy resistance through combined transcriptome analysis of a panel of human GBM cell models and TCGA GBM patient datasets. By conducting an RNA interference-mediated screen of the top 12 differentially expressed genes associated with poor survival of GBM patients, we discovered exostosin glycosyltransferase 2 (EXT2) most potently to reduce cell viability and induce cell death. In addition, untargeted and targeted metabolome analyses detected that EXT2-depleted GBM cells exhibit a differential abundance of S-adenosylmethionine (SAM) as well as SAM-associated metabolites particularly in the transsulfuration pathway. Considering these metabolic changes in mitigating ferroptosis, we found lipid peroxidation increased across the panel of non-irradiated and irradiated EXT2-depleted GBM cell models. Moreover, pretreatment with the ferroptosis inducer Ferrostatin-1 reversed lipid peroxidation and counteracted EXT2 depletion-related radiosensitization. Collectively, our results uncover a novel role of EXT2 in survival of GBM cells, their radiation response and specific metabolic pathways linked to ferroptotic cell death.

Abstract No. 04

Overcoming Glioblastoma Therapy Resistance by Interfering with intercellular vascular-to-cancer crosstalk

Soniya Bastola[1,*], *Neel Sharma*[1], *Marat S Pavlyukov*[1], and *Harley Kornblum*[1]

[1]University of California Los Angeles
*Presenting author

Glioblastoma (GBM), an aggressive form of brain cancer, is characterized by extensive neovascularization. In addition to supplying blood and nutrients, vascular endothelial (VE) cells provide trophic support to GBM cells via paracrine signaling, the precise mechanisms of which are being unraveled. Our lab has recently shown that one of the standard treatments for GBM, radiation, induced a phenotypic conversion of GSCs to vascular-like cells, which in turn, provide trophic support to the remaining tumor.

In our current study, using patient-derived GBM and VE cells as well as orthotopic GBM mouse models, we demonstrated that Endocan (ESM1), an endothelial-secreted proteoglycan, promotes malignancy and radioresistance in GBM. Using Mass spectrometry analysis, we identified PDGFRA, a commonly dysregulated Receptor Tyrosine Kinase (RTK) in GBM, as a potential receptor for Endocan. Biacore experiment confirmed that Endocan binds to PDGFRA with nanomolar affinity. Consistent with this result, we demonstrated that Endocan activates the PDGFRA pathway. Subsequent downstream signaling increases chromatin accessibility of the Myc promoter and upregulates Myc expression inducing highly stable phenotypic changes in GBM cells. Inhibition of Endocan-PDGFRA signaling with ponatinib, a PDGFRA inhibitor increases survival in the Esm1 wild-type but not in the Esm1 knock-out mouse GBM model. Identification of the Endocan/PDGFRA/Myc axis demonstrates an important role of VE cells in GBM malignancy while targeting this pathway might subdue the recurrence of GBM and further highlight the importance of vascular to tumor cell signaling for GBM biology.

Abstract No. 05

Establishment of a Rodent Glioblastoma Partial Resection Model for Preclinical Testing of Local Drug Delivery Systems

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Glioblastoma (GBM) is the most common and highly malignant brain tumor in adults. Local drug delivery systems (LDDS) represent a promising treatment strategy for GBM offering various benefits over traditional systemic drug delivery. However, their success has been limited, with only a few systems finding their way into clinical application. Yet, numerous new LDDS approaches are currently being developed. Here, (partial resection) GBM animal models play a decisive role in the preclinical testing of promising LDDS approaches. However, the establishment of such models is challenging, and only a few reports detail the process. Thus, we present our results of establishing a partial resection glioma model in rats suitable for preclinical testing of LDDS [1]. Here, C6-bearing Wistar rats and U87MG-spheroids- and patient-derived glioma stem-like cells-bearing athymic rats underwent tumor resection followed by the implantation of an exemplary LDDS into the resection cavity. We used high-resolution Magnetic Resonance Imaging to reliably monitor the inoculation of tumor cells, tumor growth, residual tumor tissue, and GBM recurrence throughout our study. The unhindered release from the exemplary LDDS was verified both in vitro and in vivo using Fluorescence Molecular Tomography. Taken together, our established rodent GBM partial resection model appears to be well-suited to evaluate the efficiency of promising LDDS. By sharing our expertise in the establishment of such a model, we intend to provide a powerful tool for future testing of promising new LDDS approaches, ultimately allowing their way into clinical application.

[1] Kubelt, C., Hellmold, D., Peschke, E., Hauck, M., Will, O., Schütt, F., ... & Held-Feindt, J. (2023). Establishment of a Rodent Glioblastoma Partial Resection Model for Chemotherapy by Local Drug Carriers—Sharing Experience. Biomedicines, 11(6), 1518.

Abstract No. 06

The power of the cellular niche in governing neural stem cell behavior

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Building an organ of correct size and cell composition during embryogenesis is of critical importance for proper organism functionality. The brain displays the highest degree of cell-type diversity in the body with great regional heterogeneity arising through particular cell division and differentiation patterns of spatiotemporally defined neural stem cell populations.
This process is highly vulnerable to disruptions, e.g. oncogenic germline mutations that precipitate in brain tumors in the context of tumor predisposition syndromes. Germline mutations provide a particularly challenging setting for the precise assessment of tumor-driving mechanisms based on an all-mutant cellular context of the organism. I hypothesize a sophisticated interplay of cell-autonomous and non-cell-autonomous mechanisms to drive tumor development and growth in syndromic brain tumor patients.

My postdoctoral work uncovering distinct and sequential functions of the epigenetic regulator Polycomb Repressive Complex (PRC)2 in cortical development provides a compelling framework for this hypothesis. Briefly, I have discovered that PRC2 is not cell-autonomously required for faithful neurogenesis, but rather orchestrates neuron production on the global tissue level in a non-cell-autonomous manner. In other words, I have shown that global tissue effects mediated by a PRC2-sufficient cellular niche provide protective mechanisms to mutant stem cells and instruct normal neurogenic behavior in individual mutant cells. However, at later stages of cortical development, namely astrogliogenesis, single mutant cells do require cell-autonomous PRC2 function for proper astrocyte progenitor proliferation and astrocyte maturation. Thus, cellular state/identity and niche properties together critically govern faithful stem cell behavior.

The established experimental assays from my postdoctoral work thus provide the profound stepping stones for evaluating the instructive cues mediated by the cellular niche in syndromic patients. The deeper mechanistic understanding of tumor development arising from these experimental approaches is thus essential for the design of innovative, effective treatments in the clinics.

Abstract No. 07

Myeloid cells coordinately induce glioma cell-intrinsic and -extrinsic pathways for chemoresistance via GP130 signaling

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Chemotherapy with Temozolomide (TMZ) is a mainstay for the treatment of primary brain tumors (glioblastomas; GBM). However, drug efficacy is reduced through a profound ability of GBM cells for DNA-repair and by the blood tumor barrier (BTB), which restricts intratumoral TMZ accumulation. Coherently, GBM almost invariably relapse with fatal outcome. We here show that GBM associated myeloid cells (GAM) simultaneously induce chemoresistance on the genetic and the vascular level by activating GP130 receptor signaling, which can be addressed therapeutically. After performing transcriptomic screens with human GAMs and microglia as well as immunohistochemical inspection of human biopsies, we observed an upregulation of the mitochondrial encoded signaling peptide Humanin in GBM. Pharmacological experiments with a humanized organotypic GBM model demonstrated that the interaction of GAM with GBM cells leads to Humanin expression. Nanomolar concentrations of Humanin promoted TMZ resistance of human stem-like GBM cells via GP130 and MAPK (ERK) activation. This was confirmed through in vitro and in vivo studies, indicating that the Humanin-GP130-ERK signaling axis drives ATR dependent DNA repair. GBM mouse models recapitulating intratumoral Humanin-release exhibited vascular alterations including an increased pericyte coverage as compared to controls. High pericyte numbers were associated with poor intratumoral accumulation of blood-borne tracers, whereas GP130 blockade attenuated BTB formation and supported the outcome of GBM chemotherapy. Altogether, we describe an overarching mechanism for TMZ resistance and outline a translatable strategy with predictive markers to improve chemotherapy for GBM.

Abstract No. 08

Glial fibrillary acidic protein, neurofilament light, matrix metalloprotease 3 and fatty acid binding protein 4 as non-invasive brain tumor biomarkers

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Gliomas are aggressive malignant tumors, with poor prognosis. There is an unmet need for the discovery of new, non-invasive biomarkers for differential diagnosis, prognosis, and management of brain tumors. Our objective was to validate four plasma biomarkers – glial fibrillary acidic protein (GFAP), neurofilament light (NEFL), matrix metalloprotease 3 (MMP3) and fatty acid binding protein 4 (FABP4) – and compare them with established brain tumor molecular markers and survival. Our cohort consisted of patients with benign and malignant brain tumors (GBM= 77, Astrocytomas= 26, Oligodendrogliomas= 23, Secondary tumors= 35, Meningiomas= 70, Schwannomas= 15, Pituitary adenomas= 15, Normal individuals= 30). For measurements, we used ultrasensitive electrochemiluminescence multiplexed immunoassays. Our results showed that high plasma GFAP concentration was associated with GBM, low GFAP and high FABP4 were associated with meningiomas, and low GFAP and low FABP4 were associated with astrocytomas and oligodendrogliomas. Several prognostic genetic alterations were significantly associated with plasma biomarker levels. We found no independent associations between plasma GFAP, NEFL, FABP4 and MMP3, and overall survival. The candidate biomarkers could not reliably discriminate GBM from primary or secondary CNS lymphomas. We can conclude that GFAP, NEFL, FABP4 and MMP3 are useful for differential diagnosis and prognosis and are associated with molecular changes in gliomas.

Abstract No. 09

Activation of MAPK, mTOR and beta-Catenin in Glioblastoma and non-malignant astrocytes – Effects on TTFields Treatment in vitro

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Glioblastoma (GBM) is the most common malignant primary brain tumor in adults. Despite surgical tumor removal, radiation and chemotherapy, outcomes are mostly poor. Thus, additional therapeutic options, such as Tumor Treating Fields (TTFields), which are already included in the National Comprehensive Cancer Network (NCCN) guidelines of GBM, are urgently needed. Protocadherin Gamma C3 (PCDHGC3) is a transmembrane glycoprotein belonging to the cadherin superfamily. It is overexpressed in GBM and in brain microvascular endothelial cells (BMECs). As we recently reported, PCDHGC3 may potentially serve as a new prognostic marker in GBM. PCDHGC3-knockout leads to altered mTOR, β-Catenin and MAPK signalling pathways in GBM and BMECs. To imitate PCDHGC3-KO, we used specific small molecules (MHY1485, SKL2001 and PMA) to activate mTOR, β-Catenin and MAPK in the U87 GBM cell line and in non-malignant astrocytes. Using PCR and Western-blot, we analyzed the impact of 72 h TTFields treatment (200 kHz/1.7 V/cm) on cells stimulated by these small molecules. In U87 we observed a decrease in the Bcl-2/Bax protein-ratio after TTFields treatment compared to the TTFields-unexposed control, indicative of increased apoptotic susceptibility. This effect was independent of stimulation with small molecules. In contrast, in non-malignant astrocytes only the co-administration of MHY1485 or PMA and TTFields lead to a decreased Bcl-2/Bax-Ratio. Furthermore, we observed increased proliferating cell nuclear antigen (PCNA) mRNA expression after TTFields exposure in unstimulated and in MAPK activated U87 cells. Non-malignant astrocytes displayed no difference in PCNA mRNA expression under any treatments. Thus, these findings may contribute to a better understanding of the TTFields mechanism of action.

Abstract No. 10

Region-dependent expression of key molecules of tumor metabolism after focused ultrasound-induced mechanical ablation - an investigation in glioblastoma organoids and primary cells

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Focused ultrasound (FUS) is a new technology that enables the controlled, spatially, and temporally precise delivery of ultrasound energy to different targets. In addition to the well-known potential applications of FUS technology in the treatment of tumors, high-intensity cavitation-based mechanical FUS ablation is currently gaining importance. Due to the novelty of this technology, little is known about the effects of mechanical FUS on peri-focally located or surviving tumor cells. In this study, we initially investigated the impact of mechanical focused ultrasound (FUS) on key molecules involved in tumor metabolism within glioblastomas (GBM). GBM are highly malignant primary intracranial tumors with a fatal prognosis due to rapid relapse and resistance to chemo- and radiotherapy. We have used both patient-derived GBM organoids (GBOs) and GBM primary cells in an in vitro 3D-hydrogel culture model. Using qPCR analysis, increased expression of glucose transporter 1 (GLUT1), pyruvate kinase muscle isozyme 2 (PKM2), and monocarboxylate transporters (MCT) 1 and 4 were found in GBOs mainly in the center of the FUS-treated region. However, due to the pronounced heterogeneity of GBMs, the findings showed strong variances in some cases. With some exceptions, these results could be reproduced in patient-derived cultured differentiated GBM cells and glioma stem-like cells (GSCs). The differentiated GBM cells showed a reduced expression of GLUT1 after FUS and in the GSCs an induction of the metabolic molecules was also detectable in the marginal area of the FUS-treated region. Complex immunohistochemical staining underlined the above results with co-staining of metabolic molecules and GSC markers and those for differentiated cells in a complex region- and marker-dependent manner. In conclusion, ablation of defined focal regions by mechanical FUS appears to lead to complex region-specific regulation of key molecules of tumor metabolism of residual/perifocal GBM cells.

Abstract No. 11

Impact of focused Ultrasound-induced mechanical ablation on the Stemness and Dormancy Characteristics of residual/peri-focally located Glioblastoma Cells

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Glioblastomas (GBMs) are highly malignant primary intracranial tumors in adults with a pronounced intra- and intertumoral heterogeneity leading to e.g., evasion from suitable treatment schedules. To overcome the heterogeneity-based highly adaptive behavior of GBMs, several potential therapeutic modalities for GBMs were investigated, but so far without significant improvements or even curative treatment options for the affected patients. Therefore, the present study evaluates the effects of mechanical cavitation-based focused ultrasound (FUS) on GBM patient-derived samples as a novel therapeutic approach in an in vitro 3D-Hydrogel culture model. Special emphasis was directed toward investigating the stemness and dormancy characteristics of GBM cells, given their pivotal involvement in both recurrence and resistance to treatment. A cytotoxicity assay revealed increasing numbers of dying GBM cells when FUS settings with increasing average incident power were used. Using qPCR analysis, increased expression of dormancy and stemness markers was detected in a complex region- and marker-dependent way, suggesting an effect of mechanical FUS beyond the focal region. Accordingly, an extreme limited dilution assay (ELDA) led to an increased ability of residual/peri-focal, formerly differentiated patient-derived GBM cells to form stem-like cell typical spheres when cultured under stemness conditions. Moreover, residual/peri-focal GBM cells were characterized by a higher resistance to temozolomide (TMZ) resulting in both lower numbers of dead cells compared to sole TMZ treatment and increased expression of dormancy- and stemness-associated markers in treated patient-derived GBM cells. Summarized, ablation of defined focal regions by mechanical FUS seems to result in the regulation of dormancy and stemness properties of the residual/peri-focally located GBM cells in a sophisticated region-specific manner.

Abstract No. 12

Activation of Wnt/β-catenin signaling is critical for the oncogenesis of choroid plexus tumors

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Choroid plexus is a secretory epithelial structure located in all brain ventricles. Choroid plexus tumors (CPTs) are rare tumors predominantly occurring in young patients with intensified malignancy in children. CPT treatment is hindered by insufficient knowledge of the tumor pathology and limited availability of valid models. By analyzing genomic and transcriptomic data from CPT patients, we discovered that large-scale chromosomal instability events of the CPT genomes cause a constitutive activation of Wnt/β-catenin signaling in human CPTs. These data were validated in CPT patient samples using molecular and histopathological methods. Pharmacological inhibition of Wnt/β-catenin signaling showed that CPT-derived cells depend on autocrine Wnt/β-catenin for survival. Additionally, constitutive Wnt/β-catenin pathway activation, either through knock-out of the negative regulator APC or overexpression of the ligand WNT3A, induced tumorigenic properties in 2D in vitro models of choroid plexus cells. Systematic hyper-activation of Wnt/β-catenin pathway in choroid plexus organoids led to reduced differentiation of choroid plexus epithelial cells, rendering them increasingly susceptible to tumor development. Remarkably, CRISPR-Cas9 knock-out of APC in choroid plexus organoids was sufficient to induce oncogenic transformation, resulting in neoplasm with features resembling supratentorial pediatric high-risk choroid plexus tumors. In summary, our study identifies Wnt/β-catenin signaling as a critical driver of CPT oncogenesis and provides the first 3D in vitro model for future pathological and therapeutic studies of CPT.

Abstract No. 13

Gamma Delta T cell recognition and activation potential in Medulloblastoma

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Medulloblastoma (MB) is a heterogeneous group of tumors developing in the cerebellum and is one of the most common malignant brain tumors in children. Fatal left untreated, the standard therapies for MB involve surgery, chemotherapy, and irradiation (only for >5 years old). Despite an overall good 5-year survival rate around 70%, first line treatment often results in severe neurological and endocrine deficits in the developing brain. Thus, there is a strong need to identify less toxic and more efficient therapeutic strategies. The emergence of cancer immunotherapy has revolutionized cancer treatment, including immune checkpoint blockade, CAR-T therapy, and infusion of T cells or NK cells. Gamma Delta (γδ) T cells, a non-conventional T cell population, are in the spotlight as a novel cancer immunotherapy strategy due to their advantageous combination of non-alloreactivity, a strong tumor cell lysis potential and a broad antigen recognition. However, their ability to target and eliminate MB cells is poorly understood.
To explore the possibility of using γδ T cells to recognize and target MB we have ex-vivo expanded different human γδ T cell subpopulations and tested their ability to target a panel of MB cells. In addition, we have characterized the expression of γδ T cells ligands on both MB cells and in MB patient datasets. We identified Ephrin-A2 receptor and the phosphoantigen/Butyrophilin complex as ligands of interest in triggering respectively Vγ9Vδ1 and Vγ9Vδ2 T cell activation leading to MB cell lysis both in monolayer and spheroid models. Preliminary results have shown that differentiated neurons and neuroepithelial stem cells, generated from IPS cells, are not targeted by γδ T cells, demonstrating the safety of this approach. The optimization of MB cell killing by γδ T cells aim to propose a novel therapeutic strategy for MB patients, with the possibility to expand to others pediatric brain tumors.

Abstract No. 14

Analysis of somatic mutations in patient-derived glioblastoma tissues and tumoroids

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Background:
Glioblastoma (GBM) is the most frequent malignant brain tumor. GB patients' median survival is <24 months, and there is no cure. The Glioma-PerMed is a consortium committed to establishing rapid and efficient glioma invasion assays and machine learning algorithms for predicting the GBM invasion in ex-vivo, personalized diagnostics, drug discovery, and patient follow-up in clinics. As a part of this consortium, we characterize the mutational landscape of GBM tissues and tumoroids.
Methods:
Five GBM tissues and seven tumoroids were generated from patient biopsies and characterized for their microenvironments, primary cilia, and proliferation. Sequencing of the obtained biological samples was done at the Latvian Biomedical Research and Study Center using MGIEasy Exome Capture V4 Probe Set and MGIEasy Exome Universal Library Prep Set on DNBSEQ-G400 sequencer. Exome sequencing reads were trimmed using fast v0.23.4; afterward, reads aligned to human genome reference GRCh38.p14 using bwa mem v0.7.17. Tumor tissue - tumoroid sample pair variant differences were detected with Strelka v2.9.10.
Results:
We sequenced five GBM tissue and tumoroid matching pairs and two independent tumoroids that did not have initial tumor sample tissue. GBM tissue samples contained 149124 to 209524 variants, while tumoroids ranged from 18169 to 186502. Specifically, we assessed the presence of mutations in 79 genes related to GB pathogenesis. In tumor tissue, we found mutations in 30-34 genes, while in tumoroids, this varied from 16 - 45 genes.
Conclusion:
In our sample set tumor tissues, most often we detected mutation in known glioma associated genes, for example, TP53, PDGFRA, ATR, and less number of cases IDH1, EGFR, and PTEN mutations.

Grants: ERA PerMed ES RTD/2023/20

Abstract No. 15

to germany empassy in iraq - baghdad

WALEED JUMAILI[1]

[1]HOSPITAL

Dr Waleed Jumaili iraqi
passport number A12294032
We Invite you to attend this seminar
in 23 may 2024

Abstract No. 16

The Interplay of Proliferation and Migration in Glioblastoma

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Glioblastoma (GBM) is the most aggressive form of primary brain tumors, characterized by high proliferation rates and infiltrative growth. In recent years, evidence was gathered that not only cell density, but also mitosis events influence the (local) migration of cells. During the process of mitotic rounding and subsequent post-division spreading, cells exert forces on their surrounding neighboring cells, influencing their shape and movement. Yet, this effect has almost exclusively been studied in healthy epithelial cell populations.

Here, we studied the effect of approximately 134,000 cell division events on the local velocity of four different glioblastoma lines under confinement. Pre-mitotic contraction and post-mitotic expansion significantly increased the migration speed of not only the cells next to the dividing cell but also their subsequent neighbors. Of note and in a characteristic temporal manner, cells responded faster to the contraction of dividing cells compared to its subsequent expansion. Estimating the importance of the interplay between proliferation and migration, an average of 20-30% of all cells were affected by these events. Furthermore, the area affected by mitosis events decreased with increasing cell density, but the average number of affected cells increased. Interestingly, the time at which the maximal effect of contraction or expansion was reached showed a negative correlatation with cell density, implying that cells react faster to cell density fluctuations in their vicinity in case of high cell densities. The density dependent reaction time of cells to mitosis could not be explained by bulk biomechanical properties. In summary, the proliferation and migration seem to be coupled in GBM in a cell density dependent manner which might be important in vivo too.

Abstract No. 17

De novo-designed binder-based CAR T cells enable effective targeting of glioblastoma

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Glioblastoma is a devastating disease with poor responses to all current therapies. Chimeric antigen receptor (CAR) T cell therapy has achieved impressive success against hematological malignancies but produces responses in only a limited number of glioblastoma patients. Here, we developed a novel platform for CAR constructs using de novo designed protein binders to target tumor antigens. We firstly designed a binder CAR targeting epidermal growth factor receptor (EGFR), which is highly expressed in glioblastoma, to validate the feasibility of our platform. Our EGFR binder CAR design promoted CAR T cell proliferation, cytotoxic cytokine secretion and exhaustion resistance, leading to better antitumor performance both in vitro and in vivo. Bulk and single-cell transcriptional profiling of binder CAR T cells revealed an underlying mechanism involving enhanced effector activity and reduced exhaustion responses. Mechanismly, the binder CARs exhibited higher surface expression and greater resistance to degradation triggered by tumor antigens. Moreover, we designed and engineered a novel CD276 binder CAR T cell product and verified its antitumor function, further demonstrating the general applicability of our binder-based CAR platform. Overall, our study provides an alternative avenue for the design of a CAR antigen-binding domain to potentiate CAR T cell antitumor efficacy.

Abstract No. 18

Effects of glucose starvation on glioblastoma cells.

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Glioblastoma (GBM) is a fast-expanding and aggressive brain tumor with a median survival of approximately 14 month. The poor prognosis is largely due to cellular invasion, which enables escape from resection and drives inevitable recurrence. Furthermore, the inability of the brain to store glucose effectively makes glucose being the most widely available nutrient in the brain microenvironment. Since tumor cells are highly dependent on glucose and high levels of glucose have been linked to increased tumor invasion and poor patient survival, the central aim of this study was to obtain a more comprehensive understanding of glucose contribution to processes associated with tumor spreading. We examined the effects of high glucose (4.5 g/l) and glucose starvation (0.1 g/l or 0.3 g/l) on two different GBM cell lines, namely U138 and LN229. Changes in collective migration, cell proliferation, cell-cell adhesion, morphology, and mRNA and protein expression of genes responsible for GBM progression were evaluated after glucose starvation over time. A significant difference was observed after treatment in both U138 and LN229 cell lines in collective migration, but not in single cell migration. After 50 h the speed in LN229 increased significantly for low glucose concentration. In contrast, U138 showed initially a decreased speed but exceeded control conditions after 60 h for low glucose concentrations. Assessing cell-cell adhesions via ß-catenin labeling showed no significant changes for U138, whereas for LN229 increased staining levels were found. Furthermore, the number of cell division and the number of BrdU (S-Phase), but not Ki67 (non-G0) positive cells was significantly decreased in low glucose groups in both cell lines. Taken together, glucose deprivation in LN229 and U138 cell lines changed tumor features essential for GBM progression. A thorough understanding of the metabolic traits that define invasive GBM cells may provide novel therapeutic targets.

Abstract No. 19

Investigating TTFields-induced alterations in the blood-brain barrier (BBB) using an ex vivo 3D glioblastoma (GBM)-BBB model

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Tumor Treating Fields (TTFields) are alternating electric fields of low intensity (1-3 V/cm) and intermediate frequency (100-500 kHz). The effects of TTFields are frequency-dependent. Administration at 200 kHz is approved as therapy for glioma WHO grade 4, specifically glioblastoma (GBM). Applied at 100 kHz, TTFields increase blood-brain barrier (BBB) permeability by de-localization of tight junction proteins claudin-5 and ZO-1 in endothelial cells. Since TTFields permeabilize the BBB reversibly, they could be used to improve drug-delivery for the treatment of brain tumors, without damaging the BBB long-term.
An *ex vivo* 3D GBM-BBB model composed of U87 GBM spheroids seeded on top of murine brain slices, which were cultured on top of immortalized murine microvascular cerebellar endothelial cells (cerebEND), was treated with 100 kHz TTFields for 72 h followed by an additional 72 h TTFields application at 200 kHz with or without the chemotherapeutic agent paclitaxel (PTX). PTX does not pass the BBB and therefore was used to test for BBB weakening, its potential to reach the GBM and to act in conjunction with the therapeutic TTFields frequency. Live/Dead staining was conducted to check for tissue viability. Trans-endothelial electrical resistance (TEER) was measured for examining BBB integrity over the treatment course. Spheroid size was measured before treatment, after 72 h TTFields at 100 kHz, and after adjacent 72 h TTFields at 200 kHz. No-treatment served as control.
The tissue was viable for the duration of the experiment. Compared to the control, the size of the GBM spheroids decreased significantly after application of TTFields at 100 kHz, as well as at adjacent 200 kHz, independently of PTX treatment during the 200 kHz TTFields application. TEER measurement confirmed increased permeability of the BBB after 100 kHz TTFields.
These results are valuable findings, which could ultimately lead to a better treatment approach of GBM.

Abstract No. 20

TAMEP progenitors shape neoplastic angiogenesis and promote glioblastoma growth

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Tumor associated myeloid cells (TAM) can have profound impact on neoplastic disease in the brain. However, in glioblastoma (GBM) TAM represent a very heterogeneous cell population and there is currently no consensus which TAM subset may be therapeutically targeted to support GBM care. Recently, we characterized a previously unacknowledged population of tumor-associated cells with a myeloid-like expression profile (TAMEP) in mouse and human GBM. The occurrence of TAMEP in human GBM was independently confirmed. Remarkably, myeloid-like, differentiated TAMEP were locally derived from SOX2 dependent progenitors, clearly separating TAMEP from brain endogenous or bone marrow derived myeloid cells. Here, we combined orthotopic GBM models with transgenic lineage tracing models allowing the in situ identification of TAMEP and quantified cell subsets over time. We noted that TAMEP progenitors were maintained throughout GBM progression while differentiated TAMEP were a transient cell subset. In lineage manipulation paradigms we found that ablating TAMEP progenitors reduced tumor vascularization and attenuated GBM growth. Abrogation of differentiated TAMEP altered vascular patterning. Altogether, TAMEP control GBM vascularization and emerge as a GBM specific therapeutic target.

Abstract No. 21

Regulation of membrane tension by Plexin receptors facilitates diffuse invasion of GBM

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Glioblastoma (GBM) is a malignant brain tumor that is characterized by uncontrolled invasive growth. Invasive GBM cells experience physical constraints and face severe mechanical challenges when they squeeze through tight interstitial space, but how GBM cells adapt to constricted space to achieve diffuse migration is not well understood.

Here, we demonstrate how GBM cells usurp transmembrane receptor Plexin-B2 to gain increased biomechanical plasticity for polarized migration through confined space. Plexins have been long known as receptors for Semaphorins to direct axon guidance. Our studies have established now a new biomechanical paradigm of cancer cell migration, with Plexins taking a key role in adjusting the membrane tension of cancer cells invading through confined space.

We demonstrated by application of a series of biomechanical measurements and imaging assays, including atomic force microscopy, optical tweezers, and Flipper-TR fluorescence lifetime imaging, a key role of Plexin-B2 in controlling membrane tension and cortical actin contractility of GBM cells, which was associated with changes of the inner membrane electrical surface charge.

Using live-cell imaging to track GBM cells negotiating microchannels, we revealed active endocytosis at cell front and filamentous actin assembly at rear to propel GBM cells through constrictions, and that these two processes are interconnected and governed by Plexin-B2 that orchestrates cortical actin and membrane tension. Our studies also indicate that Plexin-B2 has an intrinsic mechanosensitive function, responding to lateral membrane compression forces through its extracellular ring structure. Blocking the bendability of the ring by specific point mutations affected membrane internalization, permeability, phospholipid composition, as well as inner membrane surface charge.

Together, our studies unveil that Plexins play a key role in regulation of membrane tension that enables GBM cells to adapt to physical constraints and achieve polarized confined migration and define a novel therapeutic target to curb the invasiveness of GBM.

Abstract No. 22

BAG3 as a regulator of stemness and cilia homeostasis in glioblastoma

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The multidomain protein BAG3 plays various roles in promoting cancer development and is frequently overexpressed in malignant diseases, including glioblastoma (GBM). Recently, we proposed that BAG3 acts as a novel regulator of cilia homeostasis, while BAG3 knockouts (KO) of the two GBM lines U343 and U251 exhibited enhanced numbers of primary cilia. This finding is underscored by a protein-protein interaction analysis of U251 GBM cells grown either in adherent culture or as stem-like spheres. Our results suggest that BAG3 may play a role in regulating cilia assembly (ACTR3, ARL3) in adherent cells and on the other hand in sphere-cultured cells in regulating stem-like cell properties by influencing stem cell markers (SOX2, OLIG2, NES). By using different BAG3 deletion constructs, we found that only the WW domain of BAG3 is required to suppress cilia formation in GBM cells. Additionally, we observed that the WW mutant was unable to rescue nuclear translocation of YAP1, indicating a link to the Hippo pathway. Depletion of BAG3 led to a decrease of the expression of the YAP1 downstream targets AURKA and PLK1. Further the effect of BAG3-KO on cilia homeostasis could be mimicked by specific inhibitors of YAP1, indicating a regulatory effect of BAG3 via Hippo-mediated modulation of YAP1 activity. Collectively these results highlight the intricate network of BAG3 interactions in the regulation of cilia homeostasis, affecting processes related to the formation and degradation of cilia.

Abstract No. 23

Implications of RAD52 as a modulator of genomic instability in Glioblastoma Tumours

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Glioblastoma (GBM) represents the most prevalent and lethal primary intrinsic brain tumour. Although radiotherapy is the most effective non-surgical GBM therapy, recurrence is universal, in part due to self-renewing, highly tumorigenic Glioblastoma Stem Cells (GSCs, CD133 + cells) population. Targeting GSCs remains a challenging task because of their unique biology and dependency on vital survival pathways, which have equal importance for the maintenance of normal stem cells and progenitors. Thus, although glioblastoma's therapeutic resistance has been the subject of several studies, very little progress has been made in the past 30 years and the survival of these patients remains poor with a median of 15 months despite maximal therapeutic intervention. As current therapies offer only limited survival benefits, the identification and validation of new approaches in glioblastoma management is of the highest importance.
In the investigation of genomic instability in GBM, we have found increased levels of DNA:RNA hybrids, termed R-loops, in our primary GBM cells. R-loops are susceptible to DNA damage in the form of lesions and transcription errors. R-loops can impede replication fork progression, cause replication stress, induce DSBs and increase genomic instability. RAD52 is one of the key homologous recombination (HR) proteins. RAD52 binds to single-strand DNA (ssDNA) and so plays a crucial role in most HR events as well as the restart of collapsed replication forks in response to oncogene-induced replication stress. Therefore, our hypothesis points to RAD52 as a critical factor for restarting replication forks and promoting the resolution of persistent R-loops in these Replication-Transcription Conflicts, avoiding GSCs apoptosis and promoting therapeutic resistance. Thus, disruption of the resolution of RTCs, which provide adaptation to the genotoxic therapies for survival, will unravel novel druggable targets and overcome GBM therapeutic resistance and recurrence.

Abstract No. 24

Development of an ex vivo whole brain slice glioblastoma model

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Organotypic cultures of brain slices are well-established and important 3D models in neuroscience, especially in cancer research. Unfortunately, many 3D models require adult animals or focus on distinct parts of the brain, e.g. the hippocampus. Therefore, we established a whole brain slice model that conserves the complex cellular brain-architecture with its different cell types. In addition, we reduce required animal numbers by sectioning the brain of 6-to-9-day postnatal old mice into several adjacent 300 µm thick slices. These slices were cultured *in vitro* at least one week under stable conditions as carrier tissue for tumor cell spheroids and used as an *ex vivo* glioblastoma (GBM) model. To mimic tumor growth and invasion, spheroids of rat 9L/lacZ or human U87-MG GBM cells were seeded onto the murine slices. To visualize and quantify the viability of the model we used two different methods based on fluorescence signals. The AlamarBlue® assay helped to choose the optimal culture medium and to constantly observe viability over a period of 10 days. The complex structures of the brain slice and tumor invasion were visualized by life-death -staining up to 14 days after slicing. Both methods showed that the slices were viable and stable for one week, before the viability slowly decreased. Co-cultured with GBM spheroids, the viability of the slices slightly decreased, probably due to tumor invasiveness, destroying the complex brain structures and cell network of the slices. In summary, our *ex vivo* whole brain slice GBM model preserved the complex organotypic brain and tumor structure, which is necessary to achieve a solid GBM 3D model *in vitro*. As the model was stable for at least 7 days and can be cultured for longer duration, it will be useful in brain cancer research.

Abstract No. 25

BRAT1 a new therapeutic target for glioblastoma

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Glioblastoma (**GBM**), the most common and malignant primary brain tumor in adults, presents challenges due to its profound radioresistance and infiltrative growth. Despite aggressive treatments, curative therapies remain elusive. Here, we identified the protein breast cancer type 1 susceptibility protein (BRCA1)-associated Ataxia telangiectasia mutated (ATM)-activator 1 (**BRAT1**) as a novel factor driving key features of GBM, including an enhanced DNA damage response, tumor migration and invasion. Genetic BRAT1 depletion in GBM cell line U251 and glioma stem-like cell line NCH644, indicated that BRAT1 is needed for a timely repair of DNA double-strand breaks, while sensitizing the cells to radiation treatment. Further, we implemented the usage of the novel BRAT1 inhibitor **Curcusone D** for *in vitro* transwell migration and sphere migration assays, demonstrating that tumor migration/invasion of glioma stem like-cells is significantly reduced, both after pharmacological treatment and genetic BRAT1-depletion. Our recently established OTCxLSFM *ex vivo* tumor growth assay model (Haydo *et al.* 2023) was then used to show that BRAT1 inhibition combined with irradiation can synergistically inhibit tumor growth, with light sheet-microscopy revealing that tumor cell infiltration into the brain tissue is drastically reduced after treatment. In addition, the combined treatment even leads to the complete elimination of more than 30% of the tumors. In line with these findings, our *in vivo* data obtained in orthotopic GBM mouse models confirmed the essential role of BRAT1 in promoting tumor growth/invasion. To further assess global changes evoked by BRAT1-depletion and pharmacological inhibition in GBM, a series of proteomic analyses were performed. Pathway analysis confirmed the role of BRAT1 in modulating cell migration and invasion, warranting further detailed investigation. Collectively we identified BRAT1 as a novel key player of the aggressive pro-migratory/invasive phenotype of GBM and therefore identified Curcusone D as an interesting potential drug candidate for development of targeted GBM therapy.

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Abstract No. 26

Local chemotherapy of glioblastoma using RENACER® - a non-toxic and fully resorbable fibrous drug delivery system

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Background
Due to the unfavorable prognosis of glioblastoma (GBM) patients and severe side effects of systemic chemotherapy in the postoperative GBM-treatment, the feasibility of local drug application is becoming increasingly important. Such drug delivery systems (DDS) would allow to utilize agents that usually cannot cross the blood-brain barrier. Clinical observations have shown that recurrences mainly form at the resection walls after GBM extirpation. Therefore, the authors pursue the strategy of inhibiting recurrence by lining the resection cavity with a biocompatible drug-loaded fleece based on silica fibers (RENACER®), which is 100% degradable into natural *ortho*-silicic acid (oSA). Thus, in addition to the synthesis and production of this fibrous drug reservoir, degradation and *in-vitro* cytocompatibility is presented.

Methods
The RENACER® DDS was prepared by sol-gel synthesis of silica precursors and subsequently processed into μ-fibers by pressure spinning. The resulting fleeces were characterized by bending test and digital microscopy. Dissolution of the fibers was verified by a degradation test based on DIN-ISO-38405-21 for the detection of oSA. Cytotoxicity was tested on primary skin cells as well as GBM cell lines in oSA enriched cell culture medium based on DIN-EN-ISO-10993-5. The capability of lining the resection cavity and the adhesion of the DDS was demonstrated in an *ex-vivo* experiment using porcine brain. Release kinetics were determined by UV-Vis.

Results
Fleece properties were tunable in terms of flexibility (24 to 0 mm diameter at break), layer delamination and cross-linking. oSA was identified as its degradation product (complete degradation *in-vitro* after 7 to 35 days), which did not show any cytotoxicity in the *in-vitro* cell culture experiments. Finally, while drug release could be demonstrated, the fleece showed good resection cavity lining.

Conclusion
Our DDS is fully biodegradable, enabling drug release in the resection cavity and thus may be a useful future tool for improved GBM-treatment.

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Abstract No. 27

Fatty Acid Synthase and lipophagy as therapeutic targets of glioblastoma

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Inhibition of fatty acid synthase (FASN) gained significant attention as a potential anti-cancer strategy to prevent de novo synthesis of FA. However, reutilization of lipids from lipid droplets (LD), that are particularly abundant in glioblastoma (GBM), is a putative escape mechanism from lipid shortage. The novel and specific FASN inhibitor TVB-2640 (Denifanstat) recently entered a phase-3 clinical trial for GBM. LDs can be selectively targeted to lipophagy, i.e. autophagic degradation of LDs and reutilization of lipids by lysosomal lipases. Since GBM heavily relies on de novo FA synthesis, inhibition of lipophagy appears to be a reasonable strategy to potentiate the effect of FASN inhibition.

Our project will assess the anti-GBM efficacy of TVB-2640 alone and in combination with autophagy inhibition (SAR-405, chloroquine [CQ], genetic depletion of ATGs) in vitro, ex vivo and in vivo and study the effects of TVB2640 on lipidomic and metabolomic profiles in GBM. Initial in vitro FACS and MTT-assay data suggest a strong synergy of TVB-2640 with CQ or SAR-405 co-treatment on induction of cell death in several human GBM cell lines (U251, GS3, GS5, GS8, NCH644) and murine glioma cell lines (GL261, Tu2449, Tu9648). Interestingly, GBM stem-like cells (GSCs) display an enhanced sensitivity to co-treatment of TVB-2640 with SAR-405 and CQ compared to non-GSCs. Seahorse analysis revealed drastically reduced mitochondrial respiration in highly sensitive NCH644 cells following treatment with TVB-2640. Additionally, we determined the ability of GBM cells to reutilize lipids from LDs through lipophagy by immunofluorescence (IF) with BODIPY™ 493/503 LD staining, revealing a time-dependent reduction of LDs following nutrient starvation and TVB-2640 treatment. Further steps will involve establishment of knockdown/knockout GBM cell models and ex vivo organotypic brain slice cultures to explore the role of candidate lipophagy regulators for TVB-2640 sensitivity.

Abstract No. 28

Mesenchymal stem cells (MSCs) derived factors induce chemoresistance and mesenchymalization of invasive GBM

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The accumulation of mesenchymal stem cells (MSCs) in human glioblastoma (GBM) is well established. However, the pathological impact of MSC on tumor progression can be subject to intratumoral heterogeneity. To elucidate the impact of MSC on GBM in distinct tumor compartments, we combined MR-imaging of orthotopic GBM in mice with synthetic lineage identification of tumor cells and MSC tracing. Comparing MR-detection of the main tumor mass with histopathology on the single cell level revealed that a considerable fraction of infiltrative GBM cells escapes imaging-based diagnostics. This is in agreement with the current view in clinical neurooncology that undetected (and unresected) GBM cells are a likely point of origin for tumor relapse. Lineage identification (by synthetic locus control regions; sLCR) of invasive tumor cells confirmed previous results from clinical specimen that infiltrative tumor subsets largely belong to the proneural GBM subtype. Interestingly, we also observed a smaller fraction of invasive GBM with mesenchymal features. Since GBM of the mesenchymal subtype (MES-GBM) are characterized by accelerated resistance to adjuvant treatment we reasoned that invasive mesGBM constitute an exceeding risk for GBM recurrence. In vivo experimentation showed that MSC have a short half-life in the tumor core but stably associate with invasive GBM cells. In vitro experiments with a range of human stem-like GBM revealed that MSC-derived factors induce mesenchymalization (as shown by transcriptomics and sLCR-based read-outs) and resistance to Temozolomide. In synopsis, our data indicate that MSC affect clinical prognosis of GBM by protecting invasive tumor cells from chemotherapy.

Abstract No. 29

Influence of glycation on the treatment response of glioma cells to tumour treating fields

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Glioblastoma (GBM) is a highly aggressive and invasive brain tumor. The most malignant cells use aerobic glycolysis for energy production (Warburg effect), leading to the accumulation of highly reactive by-products such as methylglyoxal (MGO). In our own preliminary work, we have shown that MGO induces glycation of cell surface proteins in GBM cells, which is associated with increased invasion.
In order to reduce the invasiveness of tumor cells, the use of alternating electric fields (Tumor Treating Fields, TTFields, Novocure), which have a selective antimitotic effect on cancer cells, has been established in recent years in addition to the well-known therapeutic pillars of radiotherapy and chemotherapy. TTFields were able to prolong progression-free and overall survival. In this study, we aim to investigate the effect of preclinical application of TTFields (Inovitro™ system) in combination with GBM cells (T89G, LN229 and U87 (WHO Grad 4)) under the influence of MGO.
The cells were pretreated with 0.1 and 0.3 mM MGO for 24h and afterwards with the Inovitro™ system for 48h and 72h. The Inovitro™ system was operated with low intensity (1-3 V/cm) and medium frequency (200 kHz). To prove the efficacy of both treatments, cell death was subsequently analysed by cell counting using the Chemometec NucleoCount. In addition, the arrangement of alpha and gamma tubulin was visualised by immunofluorescence.
This study shows that TTFields can attenuate the protumorigenic effects of glycation in GBM. Application of TTFields was able to reduce the increased proliferation of cells under 0.1 mM MGO. However, higher concentrations of MGO (over 0.3 mM MGO) are toxic in this setting, meaning they were associated with an increased rate of apoptosis and TTFields enhance this effect.

Abstract No. 30

Tracing pericyte heterogeneity in the glioblastoma vasculature

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The blood-brain barrier (BBB) is essential for maintenance of CNS physiology and a regionally heterogeneic, aberrant vascular barrier (blood tumor barrier; BTB) is also maintained in glioblastoma (GBM). The BTB can deteriorate clinical care for GBM by preventing the intratumoral accumulation of blood-borne therapeutics. Pericytes have key functions in keeping BBB integrity and it was previously proposed that tumor-derived vascular mural cells initiate the BTB. If endogenous pericytes contribute to the BTB is not sufficiently explored. Here, we leveraged the lineage tracing capacity of a range of transgenic GBM mouse models, recapitulating distinct human GBM subtypes, to identify and quantitate the contribution of tumor- or host derived mural cells to the neoplastic vasculature. Our models allow an exact classification of mural cell origin and revealed a profound and stable contribution of host derived pericytes to GBM vessels. We found that vascular patterning varied between GBM with different driver mutations. Surprisingly, we detected that host and tumor equally generated the population of intratumoral vascular mural cells without any dependence on GBM subtype. Overall, our data suggest that tumor parenchymal pericytes represent a genetically stable and therapeutically promising target for BTB modulation and improved GBM treatment.

Abstract No. 31

Integrative multiomics combined with functional pharmacological profiling in patient-derived preclinical models identifies personalized therapeutic vulnerabilities of high grade glioma subtypes

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Introduction

High grade gliomas are currently incurable with very short patient survival rates. High throughput omics profiling has drastically improved classification of gliomas and biomarker discovery for molecular diagnostics. Integration of high resolution multi-omics with high throughput functional profiling can further refine patient stratification towards effective personalized medicine approaches and improved treatment outcomes.

Material and method

We investigated a cohort of >45 well-established patient-derived tumor organoid and orthotopic xenograft models derived from high grade gliomas. We conducted an omics profiling, including transcriptomics (bulk RNA-seq), epigenomics (DNA methylation arrays) and genomics (targeted DNA-seq). 27 organoid models were functionally screened ex-vivo with personalized 203 compound libraries targeting cancer pathways and epigenetic modifiers. Additional functional profiling included 16 organoid models and 1280 diversified FDA-approved drugs. To identify patient-specific vulnerabilities, we performed detailed statistical assessment and unsupervised multi-omics factor analysis on molecular and functional profiles.

Results and discussion

Multi-omics analysis revealed a presence of diverse molecular profiles, representing various genetic, epigenetic and transcriptomic subtypes of high-grade gliomas observed in patients. Integrative data analysis using multi-omics and functional characterization identified a presence of three distinct groups with varying responses to drug treatments. In particular, we observed varying efficacy of drugs targeting histone methyltransferases and pim-kinases, which were linked to specific biomarkers at the (epi)genetic and transcriptomic levels. Additional validation includes assessment of drug efficacy in time at different concentration ranges and biomarker validation in patient tumor tissue among different patient groups based on their individual omics profiles.

Conclusion

Our findings suggest that the integration of omics and functional readouts may improve defining precision medicine treatment strategies. We provide a rationale for identifying distinct patient subgroups in the preclinical phase allowing for patient-tailored therapeutic strategies in high grade gliomas.

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Abstract No. 32

Comparison of 100 kHz and 170 kHz Tumor Treating Fields (TTFields) Frequency on Blood-Brain Barrier (BBB) Integrity in a 3D in vitro BBB Model

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The restrictive permeability of the blood-brain barrier (BBB) poses a hurdle in combatting brain cancer. Tumor Treating Fields (TTFields) are alternating electric fields of intermediate frequency (100-500 kHz) and low intensity (1-3 V/cm) which, at 200 kHz, are FDA approved for treatment of glioblastoma, and CE approved for WHO grade 4 glioma. At 100 kHz, TTFields permeabilize the BBB by delocalizing tight junction associated proteins in vascular brain endothelial cells, allowing drug delivery to peripheral brain regions. We investigated the effects of TTFields at 100 kHz and 170 kHz on a 3D human cell-based BBB in vitro model with the goal of optimizing TTFields frequency settings in cell culture conditions. Therefore, we co-cultured human brain microvascular endothelial cells (HBMVEC) on glass coverslips with human pericytes in transwell inserts, or vice versa, and subjected them to TTFields at 100 kHz or 170 kHz for 72 h. Change in BBB permeability was assessed by fluorescein permeation assays with molecular weights of 4 kDa, 40 kDa and 70 kDa immediately before and after TTFields administration. Untreated cells served as controls. Effects on junctional proteins were investigated by immunofluorescence staining of ZO-1, PECAM-1, and Claudin-5 in HBMVEC and expression levels were assessed by Western-blot. We demonstrated a consistent incline in HBMVEC permeability for molecular weights of 4 kDa and 40 kDa with a significant increase from 100 kHz to 170 kHz, and a significant increase in permeability for 70 kDa after treatment at 170 kHz. Junctional proteins were delocalized from the cell membrane to the cytoplasm in all treated samples, while their expression levels did not change. Thus, TTFields at 170 kHz showed consistent or improved effects on BBB permeabilization in vitro compared to 100 kHz.

Abstract No. 33

Inheritable myc level underlie clonal competition in a model of glioblastoma.

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Glioblastoma progression, especially in its early stages, remains poorly understood. To shed light on this process, we generated a glioma model by simultaneously transferring PDGFB and genetic barcodes into mouse brains, triggering gliomagenesis in uniquely labelled cells in vivo. This enabled direct tracing of glioblastoma evolution from the earliest possible stage. The resulting gliomas showed increasing mass over time, progressing from diffuse, low-grade masses to dense, high-grade tumors.

Surprisingly, clonal analysis revealed a dramatic decrease in clone numbers during progression, with an exponential decline in clonal diversity over time, suggesting selective expansion of a subset of original clones. Computational modeling suggested that these dynamics arise from clonal-based cell-cell competition rather than differences in cell death rates or cell cycle lengths alone. Furthermore, transplantation experiments with fully progressed tumors and a model driven by EGFRvIII showed similar patterns of clonal reduction, indicating ongoing competitive selection pressures.

To investigate the molecular basis of this competition, we performed bulk transcriptome analyses coupled with lineage tracing. Myc transcriptional targets showed the strongest correlation with clonal size imbalances, with Myc expression itself being significantly anticorrelated to clonal diversity. Single-cell RNA sequencing further revealed heterogeneous Myc expression within individual clones, suggesting a potential role for Myc in driving both inter- and intra-clonal competition. Functionally, modulating Myc expression was sufficient to drive competitive dynamics in intracranially transplanted gliomas without affecting overall growth rates, corroborating its role in clonal competition.

Our findings reveal a previously inaccessible dynamic of clonal competition during glioblastoma progression and identify Myc as a key factor driving intratumoral selection. They also show the maintenance of competitive pressures and genetic heterogeneity within dominant clones. These insights advance our understanding of the complex evolutionary dynamics shaping glioblastoma biology and illustrate the power of direct lineage tracing to elucidate tumor progression.

Abstract No. 34

Tumour Treating Fields for non-invasive treatment of brain cancer

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The current treatment for glioblastoma involves radiotherapy and temozolomide (TMZ) chemotherapy, which causes distressing side effects. Therefore, there is a need to develop Glioblastoma therapies that increase survival and have reduced side effects compared to current treatments. Tumour Treating Field (TTF) therapy is a novel treatment for Glioblastoma. TTF therapy uses low intensity (1-3V/cm), intermediate frequency (150-250kHz) electric fields that are delivered to the tumour via electrodes contained in adhesive pads stuck to the skin. Crucially, the only reported side effect of TTF therapy is contact dermatitis at the attachment site and, as the device is portable, patients can receive treatment outside of clinical environments. Currently, TTF therapy is only approved in combination with TMZ treatment. This study aims to improve the efficacy of TTF therapy so that TTF may be used as a sole treatment. Drosophila melanogaster embryos were used to study the anti-cancer effects of TTF in vivo and optimise TTF parameters (frequency, voltage intensity, phase, field direction and electrode shape/position). Using live confocal microscopy we identified abnormal mitotic phenotypes that are induced by application of TTF. Using newly established parameters we are able to arrest or severely disrupt cell divisions in living tissues. Currently, we use the novel parameters to try to prevent brain tumour growth in living Drosophila larvae. We examined the effects of TTF on the nervous system and found changes in the rate of muscle contraction during active radiation. However, these defects are reversible upon field removal. Using U87 glioma cancer cells we investigated the effects of optimised TTF therapy on proliferation and cell death. Future work will continue our investigation of TTF on live firing in Drosophila neurons and the application of TTF to a Drosophila brain tumour model.

Abstract No. 35

Development of a reversible quiescence model to identify signals enabling glioblastoma cancer stem cell transition between active and quiescent cell states.

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Glioblastoma (GBM) are the most common malignant brain tumours in adults and harbour a subpopulation of cancer stem cells with tumourigenic ability and high resistant to conventional therapies. GBM stem cells (GSCs) can enter a non-proliferative reversible quiescent state, from which they re-enter the cell cycle to re-initiate tumour growth. Quiescence is a heterogenous state with different depths, defined as 'shallow' and 'deep', depending on the rate at which cells re-enter the cell cycle. We are developing a model to induce GSCs into a reversible quiescent-like state, using Bone Morphogenetic Protein 4 (BMP4) with or without Fibroblast Growth Factor 2 (FGF2) to model different depths of quiescence. BMP4 treatment with/without FGF2 induces GSCs into a quiescent-like state, shown by a dramatic reduction in proliferation and increase in p27 expression, a marker of cell cycle arrest. Stemness marker expression is retained, although strength decreases, and neuronal differentiation does not occur, but glial levels increase. Withdrawal of BMP4 and re-exposure to growth factors (GFs) causes GSCs to re-enter the cell cycle, stemness marker expression to return to control levels and GFAP expression decreases. In contrast, longer BMP4 treatment with/without FGF2 induces a deeper quiescence-like state, shown by an extended period until reactivation after re-exposure to GFs. An unbiased proteomic screening of quiescent-like and reactivating GSCs is ongoing to identify signals regulating these cell states and enabling transition. Understanding and targeting the underlying pathways to prevent GSCs entering a quiescent state, or otherwise locking them in dormancy, has the potential to half growth or eradicate the whole tumour and prevent tumour recurrence.

Abstract No. 36

NKG2C/KLRC2 TUMOR CELL EXPRESSION ENHANCES IMMUNOTHERAPEUTIC EFFICACY AGAINST GLIOBLASTOMA

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NK cell receptor proteins such as NKp, NKG2, or CLEC are highly relevant to cold tumors including glioblastoma (GBM). Here, we aimed to characterize the expression of these receptors in GBM for gaining insight into their potential role as modulators of the intratumoral microenvironment. For that, we performed a transcriptomic analysis of several NK receptors with a focus on the activating receptor encoded by KLRC2, NKG2C, among bulk and single-cell RNA seq GBM datasets. We also evaluated the effects of KLRC2-overexpressing GL261 cells in mice treated with or without PD-1 mAb. Finally, we analyzed samples from two clinical trials evaluating PD-1 mAb effects in GBM patients for determining the potential of NKG2C to serve as a biomarker of response.
As expected, several inhibitory NK receptors were expressed in GBM-infiltrating NK and T cells. However, we also demonstrated, for the first time, a strong expression of KLRC2 in tumor cells with a predominance in the infiltrating margins. Neoplastic KLRC2 expression correlated with a reduction in the number of MDSCs and with a higher level of tumor-resident lymphocytes. A stronger anti-tumor activity after PD-1 mAb treatment was observed in NKG2Chigh-expressing tumors both in mouse models and GBM patients whereas the expression of inhibitory NK receptors showed the inverse association.
Our results highlight the relevance of characterizing the macrophage population and the repertoire of NK receptors, particularly NKG2C, to identify gliomas with a higher probability of responding to immune checkpoint regulators. Besides, we propose that the extent of resection and the spatial distribution of the proposed biomarkers should be considered to decide which GBM patients could have a more favorable response to these immunotherapies.

Abstract No. 37

Brain tumoroids: patient's avatars for treatment prediction and drug development

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The modeling of tumors has become an essential and instrumental support for research. Patient avatars, crucial tools in the advancement of personalized medicine and the discovery of novel therapeutic interventions, hold immense promise. We developed a step-by-step protocol for the culture of viable and standardized tumoroids. Tumor samples are automatically minced, selected, and pieces of tumors were cultured under agitation. We performed multiplexed spatial imaging to characterize tumoroids cellular composition and organization. We used methylome profiling and cytometry to determine the molecular signature and the quantity of immune cells throughout tumoroid culture, in comparison to the parental tumor. Correlation of tumoroids generation with clinical data allowed the identification of criteria of success. Finally, we tested whether tumoroids could reflect patient treatment response by applying a STUPP-like protocol, and were suitable to discovery new treatment by testing combinatory regimens. This protocol is a cheap, easy, fast, automatized, reproducible and efficient method to generate any type of brain tumoroids. It preserves native cytoarchitecture, cellular and molecular heterogeneity and specificities. Moreover, this model mimics patient's treatment response and is functional as we identified efficient combinatory treatments. This tumoroid model represents a robust model for biological studies and new drug testing. These tumor avatars open perspectives for the establishment of a personalized medicine.

Abstract No. 38

Tumor metabolic signatures in adult-type diffuse gliomas

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Background: The mutation status of the isocitrate dehydrogenase (IDH) 1/ 2 gene is the main criterion for classifying adult-type diffuse glioma into IDH mutant-astrocytoma or -oligodendroglioma and glioblastoma (IDH wild type, wt). Mutation of the IDH 1/ 2 gene results in production of the onco-metabolite α-ketoglutarate, alterations in Krebs cycle and cell energetics. We hypothesize that IDH 1/ 2 mutation leads to further metabolic differences in IDH wt and mutant diffuse gliomas ultimately affecting tumor growth and response to therapy.

Methods: Tumor tissue and matched plasma samples (n=39) were collected during surgical resection and immediately stored for metabolic analysis. Untargeted metabolomics was performed using HILIC-HRMS and all samples were measured in one batch. PeakScout and Skyline were used for metabolite annotation. In addition, a next generation sequencing panel was used to identify other genetic alterations in tumor tissue. Downstream data analysis included supervised and unsupervised methods as well as pathway analysis.

Results and conclusions: IDH wt and IDH mutant showed different metabolic fingerprints. Approximately 25% of metabolites were present at higher levels in IDH wt tumors. The most discriminant metabolites were allantoin and 2-hydroxyglutaric acid (selectivity ratio > 0.9). The most significantly enriched pathways were cysteine and methionine metabolism and central carbon metabolism ($p<0.005$). Interestingly, the levels of microbiome-derived uremic toxins, i.e., indoxyl sulphate and p-cresyl sulphate, were also significantly higher in tumor tissue of IDH wt patients ($p<0.001$). In plasma, p-cresyl sulphate ($p=0.039$) levels, but not indoxyl sulphate, were higher in IDH wt. Moreover, there was a positive correlation between p-cresyl sulphate and indoxyl sulphate levels in plasma and tumor tissue ($r=0.678$, $p<0.001$; $r=0.461$, $p<0.005$, respectively). These results show profound metabolic differences in IDH wt and mutant tumors and introduce microbiome-derived uremic toxins as potential players in metabolic reprogramming in IDH wt tumors.

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Abstract No. 39

Investigating the role of FGFR3 expression in GBM stem cells in an in vitro culture system for cellular quiescence

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Glioblastoma (GBM) heterogeneity significantly contributes to poor survival in GBM patients, highlighting the importance of exploring GBM molecular and cellular levels to understand the disease's aggressive and recurrent nature. GBM stem cells (GSCs) are heterogeneous with regards to cell cycle status and often influenced by microenvironmental factors. In GBM, elevated expression of fibroblast growth factor receptors (FGFRs) promotes tumourigenesis and tumour growth. We and others have shown that FGFR1 promotes stemness programs in infiltrating GBM cells, while FGFR2 expression is associated with GBM cells in the tumor mass. Whether FGFR3 has specific functions in GBM, remains unclear. FGFR3 expression is associated with differentiated cellular functions and negatively correlated with GBM cell cycle. Identifying the role of specific FGFRs in initiating GSC quiescence, entering into proliferation, or maintaining cell proliferation is important for understanding the phenotypic plasticity of GSCs.

The regulation of GSC quiescence can be challenging to study in vivo due to the small size of cell populations concerned and complexity of the niche environment. A culture system that induces quiescent conditions into GSCs involves replacement of growth factors EGF/FGF2 with BMP4 after 3 days. Immunostaining with selective markers reveals GSCs in BMP4 treatment induces cell cycle exit and upregulation of quiescent marker expression. Here, we employ this culture system to evaluate the role of FGFR3 in quiescent states of GSCs. Flow cytometry analysis showed that GBM cells have a larger population of FGFR3 than FGFR1 cells. FGFR3 positive cell populations retain stemness, although have slower proliferative ability compared to FGFR1 positive populations. From this, we can suggest quiescent cells express FGFR3 then switch to FGFR1 upon activation and entering the cell cycle. Using this reversible quiescent in vitro system will allow identification and validation of novel signals involved in GSC FGFR3-mediated quiescent induction or maintenance of quiescent GSC state.

Abstract No. 40

Evaluation of effectiveness therapeutics of small molecules in human glioblastoma cells

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Glioblastoma (GBM) is the most frequent malignant tumor among the tumors of the central nervous system . The therapy involves maximal surgical resection, followed by radiotherapy and adjuvant chemotherapy with Temozolomide. Despite this, the probability that the tumor will recur is very frequent. The objective of the study was to test three small molecules alternatives to Temozolomide for the treatment of GBM: Ipatasertib, Regorafenib and Selinexor. The therapeutic efficacy of these drugs was evaluated both in immortalized GBM cell lines (A172 (TP53 wildtype) and LN18 (TP53 p.Cys238Ser mutated) and in GBM cells isolated from patients treated at the Tor Vergata Polyclinic. Ipatasertib, a selective inhibitor of the PI3K/AKT pathway, inhibits the PI3K/AKT pathway and reduces cell survival both in immortalized glioblastoma lines and in one of three primary cultures of GBM isolated from patients. Regorafenib, a multikinase inhibitor directed against EGFR, is able to inhibit the MAPK pathway by abrogating the levels of both pTYR and pERK1,2 and has also been shown to be effective in inducing cytotoxicity in both cell lines and GBM cultures isolated from the three patients. Selinexor, a nuclear export inhibitor, was effective in inducing cytotoxicity in both cell lines causing a notable increase in the expression levels of TP53 and MDM2, which are therefore retained in the nuclear compartment due to the inhibition of CRM1 and perform an enhanced oncogenic action. These results show how Ipatasertib, Regorafenib and Selinexor are effective in inducing cytotoxicity with a higher efficiency than TMZ, regardless of TP53 status and MGMT methylation. This is a relevant fact as the prognosis for patients with GBM is poor due to continuous relapses. Therefore, the use of these drugs as adjuvant therapy or as an alternative to current radio-chemotherapy could represent a real benefit in terms of survival.

Abstract No. 41

Functional characterisation of the growth factor Midkine in glioma cells

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Gliomas represent the majority of all primary brain tumors. The most common as well as the most aggressive subtype, classified as WHO grade 4, is called Glioblastoma. Due to its heterogeneity and invasiveness this tumor remains difficult to treat which leads to a medium survival of only 15 months from the time of diagnosis. Therefore interest in finding new biomarkers and therapeutic approaches is rising. Midkine (Mdk), a heparin-binding growth factor, is a promising target as it is known to positively correlate with tumor growth and negatively with the related patient overall survival. The aim of this study was to further investigate the potential use of Mdk as a biomarker as well as its potential role as a new therapeutic target. So far, we have used Western blotting to determine Mdk levels in 16 different primary glioma cultures and seven glioblastoma cell lines. To analyze the functional impact of Mdk expression in glioma cells, we developed overexpression and knockout (ko) cell models. We also performed cell viability assays and Real Time Cell Analysis (RTCA) to measure the effect of Mdk ko and overexpression, and Mdk inhibitor (iMdk) and recombinant Mdk treatment on the cell growth behaviour and viability. We were able to show that the different cell lines and primary cultures differ greatly in terms of Mdk protein level. Through iMdk treatment, we observed a reduction in cell viability of glioblastoma cells with initially high Mdk quantity down to 62.41% compared to the untreated cells after 96 hours of growth. Through RTCA we detected a reduced impedance down to 44% in the ko cells and down to 22% in the cells treated with iMdk compared to unmodified cells. The presented results suggest that Mdk promotes cell growth in glioma cells and its inhibition could reduce progression of the disease.

Abstract No. 42

SMAC mimetic GDC-0152 reprograms glioblastoma immune landscape and polarizes microglia toward a pro-inflammatory anti-tumoral phenotype

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Glioblastomas are the most aggressive brain tumors in adult characterized by an immunosuppressive microenvironment. They are invaded by tumor-associated macrophages (TAMs) supporting tumor growth and treatment escape. One current therapeutic challenge is to restore an immunocompetent environment to improve treatment efficiency. A better knowledge of spatial distribution and function of TAMs is needed to design new promising therapeutic strategies.

We used small molecule SMAC mimetic GDC-0152 (SMg) which is inhibitor of apoptosis (IAP) antagonists to decipher spatial distribution and function of TAMs. By using a syngeneic glioblastoma mouse model, we performed whole mount staining and brain clearing to investigate immune cells quantity, and spatial organization within. We then performed molecular mass cytometry (CYTOF) to accurately phenotype the immune cells involved in SM response and gain functional data. We finally used human derived glioblastoma models (explants, immune cells sorting, coculture with microglia and tumoroids) to investigate the clinical relevance of our results and human glioblastoma single cell RNA sequencing datasets (scRNAseq) to decipher IAPs' expression in TAMs.

Results showed that SMg promoted immune cells CD45+ quantity and penetration within tumors including microglia. The treatment increased monocyte-derived dendritic cells, CD8EM T cells, reactive microglia while decreasing anti-inflammatory macrophages and basal microglia. Furthermore, SM treatment decreased contacts between microglia and CD8 T cells, and microglia PDL1+. Thanks to the use of human derived model, we showed that SMg increased activated microglia and that microglia SMg-treated can increase apoptosis in co-cultivated tumoroids. Moreover, scRNAseq analysis revealed that IAPs are expressed in TAM populations.

In conclusion, SMg potentiates an anti-tumoral immune response, promotes immune cell infiltration and remodel their organization within tumors. Furthermore, our results demonstrated that microglia is a key mediator in SMg response. Targeting IAPs appears as a promising therapeutic strategy to reprogram glioblastoma immune landscape.

Abstract No. 43

Drug screening and interaction with metabolism in adult-type diffuse gliomas

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Background: Functional precision medicine is the next step in individualized patient centered medicine, seeking to guide treatment based on ex-vivo drug response of patient-derived tumor cells (PDCs). In this study we aimed to evaluate the drug response (combination of potency and efficacy) to a panel of cytotoxic and targeted agents in PDCs isolated from IDH-mutant (mut) and -wild type (wt) gliomas. Next, we sought to integrate these assessed drug responses with corresponding metabolomic tissue signatures reflecting the in-vivo metabolic profile of IDH-mut and -wt gliomas to potentially uncover therapeutic vulnerabilities.

Methods: The tumor tissues of patients with glioma were used for PDC isolation prepared by mechanical and enzymatic tissue dissociation. After short-term cultivation (7-14 days) under standard culture conditions, spheroids (n=18) were dissociated into a single-cell suspension and subjected to drug screening using a high throughput screening platform. The drug panel consisted of 66 chemotherapeutics and targeted drugs. Dose response curve (DRC) fitting per drug was performed and the area under the curve (AUC) was evaluated. Corresponding tumor tissues obtained from surgical resections were analysed by HILIC-HRMS. We then correlated the metabolomic profiles with the drug response.

Results and conclusions: Significantly lower AUC values (i.e. enhanced drug response) of Panobinostat and Vorinostat (HDAC inhibitors), AMG232 and RG7112 (MDM2 inhibitors), Selinexor (exportin-1 inhibitor) and Temsirolimus (mTOR inhibitor) were observed in IDH wt gliomas (all $p<0.05$). Statistical correlation analysis between AUC values and metabolite levels showed substantially more significant correlations (criteria: $|r|> 0.5$, $p<0.05$) between drug responses and metabolites in IDH wt tumors (1329 versus 926). These results showed an association between drug response and metabolism when using a holistic approach. Additionally, metabolic pathways associated with the response of these drugs matched the main pathways affected in IDH-wt tumors, suggesting their connection with drug susceptibility.

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Abstract No. 44

Targeted Treatment of Therapy-Resistant SOX9-Positive Cells with a Suicide Gene Therapy Approach in Glioblastoma

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Glioblastoma (GBM) is an aggressive and incurable brain tumor. Despite intensive therapeutic interventions, relapse remain inevitable, leading to a poor prognosis. SOX9 is a transcription factor important in glial fate specification and neural stem cell maintenance. SOX9 has emerged as a key mediator of cancer treatment resistance, emphasising its potential as a target for preventing relapse. Here we investigate the therapeutic potential of a novel approach targeting GBM by utilizing a SOX9-directed suicide gene therapy. We studied the interplay between SOX9 and temozolomide-mediated effects in a collection of patient-derived cell lines which revealed an important link between SOX9 activation and treatment resistance. Additionally, analysis of patient tumor samples unveiled that a higher fraction of SOX9-positive cells correlated with poorer survival outcomes, further underscoring the significance of SOX9 in GBM. Expanding upon these findings, we developed a therapeutic approach utilizing a suicide gene system of Herpes Simplex Virus-Thymidine Kinase gene (HSV-TK) and the prodrug ganciclovir (GCV) specifically directed towards SOX9-positive cells through an SOX9-specific response element. We demonstrate that this gene therapy approach can efficiently eliminate tumour cells that typically evade standard care regimens in combination with temozolomide in vitro. Furthermore, SOX9-directed gene therapy prolonged the survival of orthotopic xenograft models of GBM further synergizing with fractionated radiation treatment. This study underscores the role of SOX9 in GBM and provides proof of concept of the SOX9-directed HSV-TK/GCV therapeutic effect. Clinical translation of this approach holds promise for improving the survival of patients with GBM.

Abstract No. 45

Matched primary-relapse pedHGG patient-derived xenograft models to identify therapy resistance profiles

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Background
We have established patient-derived xenografts (PDXs) from matched primary-relapse pairs of pediatric high-grade gliomas (pedHGGs) in collaboration with the Swedish Childhood Tumor Biobank. Further, we used human iPSC derived cortical organoids to evaluate interactions between glial and tumor cells under treatment pressure potentially lost in xenograft models. Using this setup, we hope to follow tumor relapse evolution and to identify novel mechanisms behind treatment evasion and relapse formation.

Methods
Tumoral single cell suspensions from matched primary-relapse pairs were injected orthotopically in immunodeficient NOG mice. We establish in vitro 2D models in stem cell conditions from the first generation of animals transplanted. Co-culture of these cells with normal cells in 3D assembloids were performed using 250-300 days old cortical organoids. Single-cell RNA sequencing and bulk RNA sequencing (RNA-seq) on patient biopsies, their corresponding PDXs from first generation of mice, and PDX-derived cell lines, was performed to track potential changes in vivo and in vitro and to further validate primary-relapse specific evolution.

Results
2D and 3D cell cultures offered efficient labeling and we quantified differences in sensitivity to standard therapy between primary and relapse samples. PDX models and assembloids highlighted pedHGG growth patterns, modes of invasion and cell interactions, especially astrocyte-tumor cell interactions. These 3D models also allow for further validation of targeted drug testing based on multiomics data (WGS, methylation and RNA-seq) from the patient biopsies.

Conclusion
PedHGG primary-relapse in vitro 2D cell line / in vivo 3D assembloid models together with PDXs is a robust approach to study different aspects of relapse-specific tumor biology. Our results offer insights aiding the development of novel therapeutics inhibiting relapse mechanisms already in the first line of treatment.

Abstract No. 46

Strategies for personalized glioma medicine

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Glioblastoma (GBM) is a highly aggressive tumour harbouring Glioma Stem Cells (GSC) which have block in differentiation with uncontrolled self-renewal property. GSCs have high neuroinvasive property in the brain which is unpredictable. GSCs express neural stem cell markers as well as adapts neurodevelopmental pathways. We hypothesize that dampening the stemness will abolish the invasion behaviour and could trigger their differentiation programs. To do this, we develop a screening system where we search for small molecules, genetic modifiers, and cellular structures that could disrupt the balance between self-renewal and differentiation. Notably, we will use a set of patient-specific GSCs for our screen. We will then test the identified small molecules and targets in a glioma-invasion assay in which we will use patient-specific glioma tumoroids and human brain organoids. Our aim is to test interventions in at least 30 different patient samples to identify the differential sensitivities of compounds across several patients. Our designed assays and readouts will advance the personalized glioma medicine.

Abstract No. 47

Tracking glioblastoma cell plasticity identifies GB-Hybrid, a therapy resistant cell state dependent on nuclear import

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Introduction:
Glioblastoma (GB), despite extensive fundamental and clinical research, still carries a fatal prognosis. Prior investigations have uncovered the existence of distinct cell states within GB tumor cells; however, the molecular mechanism underlying adaptive GB cell plasticity, that is, their potential to transition from one state to another under conventional therapy is unclear. As a consequence, the therapeutic exploitation of GB cell plasticity remains unexplored because of the lack of selective vulnerabilities. Thus, we investigated the unique characteristics and vulnerabilities of hybrid GB cells rather than focusing on those that define specific identities.

Methods:
To track changes in GB plasticity in real time, we employed patient-derived cell lines with diverse mutational and phenotypic profiles cultured as neurospheres and transduced them with two specific fluorescent synthetic reporters. These reporters were designed to label defined GB identities and have been extensively validated both in vitro and in vivo.
We functionally and transcriptionally investigated GB cells labeled with activated reporters for both the Proneural and Mesenchymal subtypes. Using a multi-level methodological strategy that includes bulk and scRNA-seq, scChIP-seq, nuclear proteomics, high-resolution imaging, orthotopic mouse models, clinical dataset analysis, and pharmacological and genetic approaches, we gained insights into the hybrid cell state.

Results and discussion:
We revealed the existence of a hybrid state, named GB-Hybrid. We showed that this aggressive cell state is induced by chemotherapy or radiotherapy, is resistant and proliferative. Importantly, the GB-Hybrid geneset signature has marked prognostic power when tested in the TCGA database. Mechanistically, GB-Hybrid cells are larger with more RNA per cell, have more open chromatin, and enhance the nuclear import/export machinery, which leads to redistribution of transcription factors and activation of Myc. Notably, pharmacological and genetic targeting of the nuclear import/export machinery hinders the maintenance of the GB-Hybrid, thus reducing proliferation and sensitizing GB cells to conventional therapies.

Abstract No. 48

Further elucidating the genetic landscape of glioma predisposition in a European cohort of familial glioma cases

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Background/Objectives: Familial occurrence of primary brain tumors, including gliomas, is observed in about 5% of cases. Studies on the genetic landscape of glioma predisposition are still scarce.

Methods: Leukocyte DNA of 214 adult glioma patients from 207 families with evidence for a tumor predisposition, i.e. tumors in the family history or a personal history of a second tumor, was analyzed by whole-exome sequencing. Data analysis was done using two approaches: (1) variants in 154 established cancer predisposition genes (CPGs) or suspected glioma risk genes were extracted and classified, (2) the frequency of rare (MAF<0.01%) or deleterious variants in all genes was compared in the glioma cohort and a control cohort (n=274 families).

Results: BRCA2 variants (n=5), including the pathogenic variants c.316+5G>C, p.(Q742*), p.(K944*), p.(I1470Kfs*11), were significantly more frequent in the glioma than in the control cohort (P=0.014). ATM variants predicted to be deleterious (n=5) were equally frequent in glioma cases, and preferentially predisposed to astrocytoma of CNS WHO grade 2-4 (astrocytoma diagnosed in 4/5 cases with versus 41/209 cases without ATM variant; P=0.007). Rare deleterious variants in APC, EGFR, DICER1, PMS2, POLE, and SDHA were recurrently observed in the glioma cohort. Furthermore, we identified five other genes, not previously associated with cancer predisposition, that were affected significantly more frequently in the glioma than in the control cohort. The genes affected by rare deleterious variants in glioma cases of our study that overlapped with those in the familial glioma cohort reported by Choi et al., Sci. Adv., 2023 were ATM, BRIP1, CDKN2A, PMS2, and POLE

(known CPGs), and KAT5, SLC4A7 and WDR7 (novel CPGs), among others.

Conclusion: Our study on a large European cohort of familial glioma cases implicates known CPGs, particularly BRCA2 and ATM, and novel CPGs in glioma risk.

Abstract No. 49

Unveiling Key Drivers of Glioma Progression: A Comparative Transcriptomic and Functional Analysis

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Gliomas, characterized by their aggressive behavior and resistance to treatment, pose a significant challenge in oncology. Understanding the molecular mechanisms that govern malignant progression is crucial for developing effective therapeutic strategies. In this study, we employed a glioma model based on retroviral transduction of PDGF-B oncogene in embryonic mice that simulate glioma progression.

The procedure generates both low-grade (LG) gliomas which exhibited an immunostimulatory phenotype, and high-grade (HG) gliomas capable of developing secondary tumors after orthotopic transplantation. These HG gliomas display an M2 pro-tumorigenic and anti-inflammatory profile promoting immune escape(1). Leveraging RNA sequencing, a comprehensive transcriptomic analysis of both LG and HG glioma samples had been conducted, to elucidate tumoral progression at the molecular level.

Through comparative analysis with transcriptomic data from human LG and HG gliomas obtained from publicly available databases, we identified key genes - Engrailed2, Hoxa5, Ulbp1, and Axl – overexpressed in both murine and human HG gliomas. To investigate their roles in glioma progression, we employed retroviral-mediated gene downregulation in our HG glioma cells, enabling us to assess the impact on cell proliferation and migration - hallmark features of high-grade gliomas. After conducting in vitro functional assays, our next step will be to evaluate the effects of silencing these genes in vivo to delineate their tumorigenic and immune evasive potential.

This integrative approach combining murine modeling, transcriptomics, and functional assays provides valuable insights into tumor progression and the dynamic interplay between gliomas and the immune system, offering potential avenues for therapeutic intervention aimed at disrupting tumoral progression and enhancing treatment efficacy.

1 Appolloni I et al., Cancer Lett. 2019 - PMID: 30312732

Abstract No. 50

Dissecting Clonal Evolution and Immune Evasion in Glioblastoma Progression

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Glioblastoma continues to be a daunting obstacle in oncology research, with its early-stage progression being particularly enigmatic. In a recent published work, we traced the clonal dynamics of Glioblastoma evolution by the simultaneous transfer of PDGFB and genetic barcodes into mouse brains. We observed a continuous clonal loss during the acquisition of a malignant phenotype, underlined by the modulation of the levels of c-Myc expression and their functional targets.
In this study, we delve deeper into the evolution of Glioblastoma by transplanting multiclonal, early-stage glioma cells into multiple immunodeficient NOD-SCID mice. This experimental design allowed us to track the clonal dynamics over serial transplants, revealing how early-stage glioma clones acquire immune-evasive features capable of initiating tertiary tumors in immune-competent hosts. Through barcode sequencing and single-cell RNA sequencing of early-stage gliomas, coupled with bulk RNA sequencing of secondary gliomas, we dissected the clonal and transcriptomic landscape of these tumors. We observed that, of the various clones composing primary tumors, just a few of them are predominating in subsequent passages. Furthermore, the clonal composition of secondary tumors derived from the same primary gliomas showed partial overlap, indicating a partial predetermination in the development of immune-evasive behavior.
Our intra- and inter-clonal transcriptomic analysis across various stages of tumor progression is shedding light on how new functional traits can emerge in gliomas. Further analysis of our data will determine whether it is the result of the expansion of clones already possessing these traits, or if they stem from functional adaptations within the clones themselves.

Abstract No. 51

Directed trans-differentiation of H3K27M-mutant diffuse midline gliomas to adipocyte-like non-malignant cells

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H3K27M-mutant diffuse midline gliomas (DMGs) are amongst the most lethal pediatric brain tumors. Patients diagnosed with DMG face a poor prognosis of 9-12 months from diagnosis. The oncohistone H3 results in hypomethylation and the subsequent cascade of tumorigenic pathways. Directed transformation of malignant cells to non-malignant cells has been shown to be effective in non-brain tumors. For example, breast cancer cells undergoing epithelial-mesenchymal transition, have been shown to be susceptible to trans-differentiation into mature adipocytes.
Given the hypomethylated state of DMGs, we hypothesize that DMG epigenome is primed for rapid transformation into mature adipocyte-like cells resulting in a non-proliferative cellular state. We thus investigated patient-derived primary DMG cells and found expression of gene regulatory programs indicative of their susceptibility to adipogenesis (eg, PPARG, CEBPB, CEBPA, RXRA, ZEB1). We established a protocol comprising of a combination of hormones, corticosteroids, and pharmacological agonists known to be involved in driving adipogenesis to induce trans-differentiation of DMG cells. DMG cells (n=3) were treated with various exposure times and pharmacological combinations followed by assessment of trans-differentiation. A fat-soluble fluorescent dye/tracer (Nile Red staining) was used to stain lipid droplets, defined as neutral lipids storage organelles, typically found in mature adipocytes. We were able to induce formation of lipid droplets in trans-differentiated DMG cells, whereas no lipid droplets formed in untreated DMG cells. Although nascent lipid droplets might be an indication of DMG cells susceptibility in trans-differentiating to mature adipocyte-like cells, another explanation for their formation might be due to the brain being the second most lipid-abundant organ after the adipose tissue. Further studies are required to better comprehend the phenotype of trans-differentiated DMG cells. Fully characterization of adipocyte-like DMGs and assessment of their resistance/response to therapy in vitro and tumorigenicity in vivo are underway.

Abstract No. 52

The role of C-terminal domain (CD44-binding domain) of Spp1 in autocrine and paracrine stimulation of murine NF1/p53-deficient glioma cells

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Osteopontin (Spp1) is a secreted signaling molecule, involved in physiological and pathological processes, including immune responses and cancer progression. Spp1 acts on cells by binding to: the integrin receptor (via the N-terminal domain of Spp1), and the CD44 protein (via the C-terminal domain of Spp1). Spp1 is overexpressed in human glioma cells, particularly in glioma stem-like cells (GSCs), which co-express high levels of CD44. We have previously demonstrated that the N-terminal Spp1 domain participates in reprogramming of glioma-associated microglia and macrophages, while the CD44-binding C-terminal domain of Spp1 is important for maintenance of self-renewal and pluripotency of GSCs. However, the specific roles of CD44-binding domain of Spp1 in glioma progression and transcriptional programs in glioma cells were not investigated. Therefore, we overexpressed the variants coding for the full-length Spp1 (Spp1-wt), or Spp1 with deleted C-terminal domain (Spp1-ΔC) in murine NF1/p53-deficient glioma cells. We assessed proliferation and invasive potential of control and modified glioma cells (BrdU assay and gelatin zymography), Spp1 expression (ELISA and Western blotting), transcriptomic changes in modified cells (RNA-seq), and changes in endothelial-mesenchymal transition (EMT) markers at the RNA and protein level. Overexpression of the Spp1-ΔC produced profound changes in glioma cell morphology, in particular detachment of cells from tissue culture plate. Transcriptomic profiles show distinct transcriptional responses to Spp1-wt or Spp1-ΔC overexpression, which permitted identification of C-terminus-dependent and independent effects of Spp1. The genes up-regulated in the cells overexpressing Spp1-wt are enriched in the genes in the KEGG pathways: “Axon guidance”, “Focal adhesion”, and “ECM-receptor interaction” which indicates glioma-neuron interconnections. We furthermore demonstrated that signaling involving the CD44-binding domain of Spp1 changes the invasive potential of NF1/p53-deficent glioma cells, as well as expression of some EMT markers. The results point to new mechanisms through which glioma-derived Spp1 contributes to glioma progression.
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Abstract No. 53

Unraveling the cellular diversity and spatial architecture of infant-type hemispheric gliomas using multi-omic profiling

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Pediatric brain tumors are rooted in genetic and epigenetic dysregulation occurring during fetal development. These tumors exhibit a maturation block resulting in a continuum of immature "progenitor-like" oncogenic states organized in a "developmental-like" cellular hierarchy. Yet, the cellular origins of pediatric brain tumors, in particular in infant-type hemispheric gliomas (IHGs), and the contribution of these oncogenic states to tumor pathobiology remain poorly understood. Focusing on IHGs, a highly heterogenous tumor entity with distinct receptor tyrosine kinase (RTK) gene fusions (ROS1, ALK, MET, NTRK), we assembled a cohort of n=27 cases and performed single-nuclei multi-omic RNA/ATAC-seq, spatial transcriptomics (MERFISH). We curated and annotated a cellular atlas with over 90'000 single-nuclei transcriptomes and 1M spatially resolved cells. Data integration with single-cell datasets from the developing brain allowed us to resolve five "progenitor-like" oncogenic states based on developmental stage (radial glia, astrocyte, oligodendrocyte, oligodendrocyte progenitor cell (OPC), neuronal), with age-specific differences. Younger patients' tumors display a bias towards the neuronal lineage, while older patient appear to be enriched by OPC-like cells. Comparisons between IHG and low-grade gliomas, as well as gliomas with histone alterations (H3K27M/H3G34R), further revealed differences in oncogenic cellular state composition, highlighting the characteristic heterogeneity of pediatric brain tumors. Using trajectory inference and gene velocity modeling, we describe a "developmental-like" and dynamic organization of oncogenic cellular states, suggesting cycling radial glial-like cells as a common cell of origin. Open chromatin fragment analysis (ATAC-seq) also indicated that cells from these oncogenic states are enriched for binding sites of transcription factors known to regulate brain cell maturation. Finally we mapped using MERFISH these oncogenic states onto tissue architecture, and resolved interactions between microglia and tumor cells. Taken together, our multi-omics atlas describes the cellular and spatial heterogeneity of IHGs and RTK-fused pediatric gliomas, advancing our understanding of their unique biology and clinical implications.

Abstract No. 54

Integrated proteomics spotlight the proteasome as a therapeutic vulnerability in Embryonal Tumors with Multilayered Rosettes

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Embryonal tumors with multilayered rosettes (ETMR) are rare malignant embryonal brain tumors harboring a poor prognosis. Due to the scarcity of these tumors, our comprehension of ETMR tumor biology is currently limited to only few previous molecular studies. In this study, we explored integrated ETMR proteomics with the aim to identify novel therapeutic vulnerabilities in these deadly tumors.
Using mass spectrometry, proteome data were acquired from FFPE tissue of 16 ETMR tissue samples and the ETMR cell line BT183. Proteome data were integrated with case-matched global DNA methylation data, publicly available transcriptome data, and proteome data of further pediatric brain tumors.
Proteome-based cluster analyses grouped ETMR samples according to histomorphology, separating neuropil-rich tumors with neuronal signatures from primitive tumors with signatures relating to stemness and chromosome organization. Microdissection analyses highlighted the close relationship of ETMR histomorphology and proteome profiles, regardless of intra- or intertumoral comparisons.
Integrated proteomics showcased that ETMR and BT183 cells harbor proteasome regulatory proteins in abundancy, implicating their strong dependency on the proteasome machinery to safeguard proteostasis. Indeed, in vitro assays using BT183 highlighted that ETMR tumor cells are highly vulnerable towards treatment with the CNS penetrant proteasome inhibitor Marizomib.
In summary, histomorphology and proteomics are closely linked in ETMR. Pervasive and histomorphology-independent abundancy of proteasome regulatory proteins indicates a strong proteasome dependency throughout ETMR. As validated in cell culture experiments, proteasome inhibition poses a promising therapeutic option in ETMR.

Abstract No. 55

Glioma-microglia crosstalk is reshaped by hypoxia at the chromatin and transcription level

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Chromatin, the assembly of DNA and its associated proteins, undergoes significant changes and often becomes dysregulated in cancers, including malignant gliomas which have a poor prognosis due to ineffective treatments. The microenvironment of glioblastoma (GBM), the most malignant glioma, is infiltrated by glioma-associated macrophages and microglia (GAMs), which promote tumour invasion and immunosuppression. GBM contains necrotic regions, characterized by hypoxia (low oxygen) - a significant factor in reprogramming the gene expression, cell invasion, stemness and impeding the efficacy of major treatments. Hypoxia affects chromatin structure, globally increases the methylation of histones and DNA; however, it is not clear how these changes contribute to the hypoxia-reprogrammed transcriptome. The effects of hypoxia on glioma-GAMs interactions are still poorly understood. Using glioma-microglia co-cultures, we determined changes in the chromatin accessibility (by ATACseq) and gene expression (by RNAseq) in individual cells imposed by hypoxic conditions. In glioma cells, hypoxia was a stronger factor in changing the chromatin accessibility than co-culture with microglia. On the contrary, the chromatin accessibility in microglia was more affected by interactions with glioma cells than due to response to hypoxia. Interestingly, the hypoxia effect dominated over the gene expression changes in both cell types, of which approximately 20% was reflected by the changes in the chromatin accessibility. We demonstrate that a transcriptional response to hypoxia dominates the gene expression in glioma-GAM co-cultures. Nevertheless, we identified differentially expressed genes altered at the chromatin and transcriptional levels in response to either co-culture or hypoxia. Those genes are implicated in cell motility, morphology or immunosuppression. Many of the changes observed in cell co-cultures were corroborated in available human datasets which warrants further work exploring these new candidate genes as potential druggable targets in glioma-GAMs interactions. Studies were supported by the National Science Center grant (Poland) 2019/33/B/NZ1/01556.

Abstract No. 56

Development of Genetically Labeled 3D Glioblastoma Models for Studying Drug Response

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Glioblastomas (GBMs) are aggressive brain tumors with a dismal prognosis, emphasizing the critical need for effective research models. Glioblastoma stem cells (GSCs) dictate tumor progression and recurrence, yet in vitro cultures often fail to accurately capture their heterogeneity. Conventional 2D cultures inadequately represent GBM invasiveness and drug-resistance due to their dependence on host brain tissue interactions and in-vitro clonal selection. 3D cultures of patient-derived spheroids (PDS) represent a promising approach for replicating in vivo cell interactions and tumor growth, serving as a robust model for assessing anti-tumoral drug responses in glioblastoma (GBM). Preserving tumor heterogeneity characteristics in GBM is a critical aspect for the reliability of such models as platforms for studying anti-cancer drugs against GBM. This project aims to elucidate the clonal behavior of GBM cells within PDS to assess how well this 3D culture model maintains the clonal heterogeneity of the cells from which it was initially constituted. A lentiviral library with a multiplicity exceeding 3x10^4 was employed to label GBM cells. These cells were cultured as spheroids in Matrigel droplets, thus clonal composition can be evaluated over-time via targeted DNA-seq.
This approach will provide a better understanding of GBM spheroids clonal complexity preservation, opening new perspectives for the development of targeted therapies and drug response, allowing the identification of resistant tumor subpopulations. The development of an experimental model that best reproduces the growth characteristics of GBM in vivo will allow the identification of new molecular targets for drugs and possible resistance mechanisms.

Abstract No. 57

Deciphering Autophagy Role in Glioblastoma Biology

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Autophagy is a lysosomal-mediated degradation pathway, which ensures cellular and organismal homeostasis and its deregulation is associated with a plethora of human diseases, especially cancer. The role of autophagy in tumorigenesis is context-dependent, as it can both suppress and promote tumor growth during tumor initiation or progression, respectively. To date, autophagy role in glioblastoma (GBM) biology is extremely controversial but an increasing number of literature data suggests an impairment of autophagy proficiency in GBM. Our aim is to characterize the status and competence of the autophagy machinery in GBM. Through in silico experiments we have identified a negative modulation of transcript levels of ULK1 and of other autophagy players in GBM patients. Moreover, we have correlated ULK1 expression levels to patient survival and we have found a poor survival rate for patient expressing lower ULK1 levels to respect patients expressing high ULK1 levels. Coherently, the protein expression levels of the autophagy initiator ULK1 result significantly down-regulated in GBM tissues compared to non-tumoral ones in western blotting and immunohistochemistry analyses. Instead, there are no significant modulation in proteins levels of the other autophagy players but a slow-down of the autophagic flux is detected, as demonstrated by the accumulation of the autophagy substrate p62. Prompted by these evidences we transiently overexpressed either ULK1 wild-type (WT) or its death kinase (KD) mutant in a continuous cell line, U373, by lipofection and we are currently evaluating cellular proliferation and migration capability in dependance of ULK1 dosage by taking advantage of proteomic analysis. Beside performing in vitro experiments in 2D models, we will hopefully investigate the effect of ULK1 dosage in more physiological 3D models.

Abstract No. 58

Personalized GBM invasion in human brain organoids and intervention strategies

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Glioblastoma stem cells (GSCs) have unpredictable neuroinvasive behavior in the brain. We can study the neuroinvasive behavior of patient-specific GSCs using human brain organoids. Depending on the genetic background and aggressivity of patient-specific GSCs, brain organoids can stratify neuroinvasion behaviors. Secondly, by analyzing transcriptomic programs, stemness, and primary cilia of patient-specific GSCs, we could identify cilia heterogeneity among patients who may rewire the signaling and genetic programs of GSCs. An integrative analysis identifies primary cilia as critical for maintaining the stemness of GSCs. By altering primary cilia dynamics, we could dampen the stemness of GSCs and trigger them to differentiate, thus reducing the invasion behavior. By applying the strategies above, we hope to study the invasion behaviors of more than 100 GBM patients, stratify them, and design therapeutic interventions in a patient-specific manner.

Abstract No. 59

Intercellular communication by extracellular vesicles in medulloblastoma: Molecular mechanisms of release and functional consequences

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Background: Tumor-derived extracellular vesicles (EVs) are key mediators of intercellular communication and important modulators of the tumor microenvironment (TME). However, little is known about their role in medulloblastoma (MB). The aim of this study is to determine regulatory mechanisms of EV release in MB and to assess functional consequences for cells resident in the cerebellar TME.

Methods: We used Nanoparticle Tracking Analysis (NTA) to quantify MB-derived EVs. We used pharmacological and genetic interference strategies to assess potential regulators of EVs. We used pHluorin_M153R-CD63_mScarlet reporter cell lines, confocal live cell imaging and scanning electron microscopy to visualize EV release and uptake into target cells. We used an image-based quantification method to quantify EV deposition. We used a 2D and 3D astrocyte-MB tumor co-culture system and cerebellar tissue slices to explore EV-dependent mechanisms of MB modulation of the TME.

Results: We confirmed the release of EVs of variable size by all MB subgroups. Released MB EVs are detected both in the supernatant as well as in matrix-attached retraction fibers. In SHH MB cells expressing the pro-migratory Ser/Thr kinase MAP4K4, EV release and deposition is repressed in the presence of the MAP4K inhibitor prostetin/12k. We identified cerebellar astrocytes as a potential cellular target population for MB-derived EVs. Finally, we identified tunnelling nanotubes (TNTs) as possible routes for EV exchange between MB tumor cells and astrocytes.

Conclusion: EVs from cultured MB cells are transferred to astrocytes. EV deposition and uptake is repressed by prostetin/12k, indicating the implication of MAP4Ks in the biogenesis and release of MB EVs. Thus, interference with EV transfer to astrocytes could represent a novel therapeutic approach in MB.

Abstract No. 60

Exploring EphrinB3 and EphA2 in glioblastoma cells after conventional radiotherapy to reveal possible sensitizing strategies

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Glioblastoma is the most common primary brain tumour with poor overall survival despite aggressive treatment. To improve patients' response to treatment, signalling pathways, connected with therapy resistance, could be targeted. It is known that the Ephrin and Eph signalling pathways are important in tumour progression; EphrinB3 controls glioblastoma invasion and promotes tumour growth, whereas EphA2 is upregulated in glioblastomas and its phosphorylation at S897 leads to migration, and invasion of glioblastoma cells.

We explored the effect of radiotherapy (RT) on a patient-derived glioblastoma cell line, focusing on EphrinB3 and EphA2 expression. Clonogenic assay and cell proliferation were studied after single dose (10 Gy) and fractionated RT (4x2.5 Gy). Differences in EphrinB3 and EphA2 S897 expression and activation of the DNA-dependent protein kinase (DNA-PKcs) S2056 were analysed by western blot.

Our results showed that RT significantly affected clonogenic potential and doubling time of glioblastoma cells in dose-dependent manner, leading to reduced number and size of colonies in RT-treated samples.

As expected, RT-induced DDR signalling immediately after irradiation in both RT procedures, leading to an increase in DNA-PKcs, phosphorylated at S2056, site, previously associated with poor clinical outcome and therapy resistance. Analysis of EphrinB3 and EphA2 S897 showed that both proteins were present in glioblastoma cells prior and post RT in both RT procedures. Although there was no major difference in EphrinB3 or EphA2 S897 expression between irradiated and control samples in case of single dose RT, we did observe 2.5-fold upregulation of EphrinB3 and 4-fold upregulation of EphA2 S897 after fractionated RT. Those results indicate that repetitive, low dose irradiation is more efficient in triggering evolution of cancer cells towards RT-resistant phenotype.

Our results show that EphrinB3 and EphA2 S897 are expressed in glioblastoma after RT, thus EphrinB3 and/or EphA2 should be explored for RT sensitizing capacity in glioblastoma.

Abstract No. 61

Mapping the brain tumor microenvironment in pediatric diffuse midline glioma using multiplex immunofluorescence and AI prediction model

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Diffuse midline glioma (DMG) is a fatal childhood brain cancer. Limited understanding of tumor microenvironment (TME) and tumor associated antigens have hindered the design of effective immunotherapies.
To investigate TME, whole brains were collected at autopsy (n=70 pediatric subjects) to assemble a tissue microarray (TMA) including DMG (n=50), GBM (n=9), ependymoma (n=5), ATRT (n=2). Brain tissue from non-malignant patients (n=10) were also obtained. Per patient, up to four brain regions (primary tumor, metastatic and adjacent healthy) were selected. Three cores were obtained per site, resulting in 918 cores. H&E and immunohistochemistry (IHC) were performed and reviewed by a neuropathologist to score tumor content. Multiplex immunofluorescence (MxIF) was used to probe 43 biomarkers on a single TMA slide, focusing on immune cells. Immune microenvironment was correlated with clinical data and patient response to treatments.
We found greater cytotoxic T-cell infiltration and activated resident microglia in DMG patients compared to other CNS tumors. Amongst DMGs, T-cell infiltration was more abundant in ONC201 treated patients who received immunotherapy. DMGs exhibited a greater number of activated microglia (Iba1+), more pronounced in primary tumor sites than metastatic locations, than CD8+ T-cells. However, DMG primary site contained higher number of immune suppressive CD163+ cells compared to metastatic sites. Further, primary DMG sites were enriched with tumor stem cells by higher numbers of SOX2, vimentin and nestin positive cells and showed enriched vascularization, VGFR2+ cells, compared to adjacent healthy sites and varied by tumor content. We further demonstrate the accurate prediction (test set ROCAUC to predict marker-positive cells 0.71 (0.48, 0.87), median (full range)) of 22 protein markers from H&E slides using a deep learning pipeline.
In summary, our TMA provides insights into immune cell infiltration, microglial activation, tumor stem cell presence, and vascularization patterns and enabels AI prediction, which advances our understanding of immunotherapies for DMGs.

Supported by SciSerTec
Web: http://www.scisertec.de

Abstract No. 62

H2A.Z histone variants facilitate HDACi-dependent removal of H3.3K27M mutant protein in paediatric high-grade glioma cells

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Diffuse intrinsic pontine gliomas (DIPG) are deadly paediatric brain tumours, non-resectable due to brainstem localisation and diffusive growth. Patients with DIPG have a dismal prognosis of 9-12 months of survival with no effective therapy. Over 80% of DIPGs harbour a mutation in histone 3 (H3.3 or H3.1) resulting in a lysine to methionine substitution (H3K27M). H3K27M causes global epigenetic alterations (a loss of H3K27 trimethylation and an increase in H3K27 acetylation) resulting in aberrant gene expression. To date, no therapeutic strategy exists to suppress the levels of oncogenic H3K27M.

We show that pan-HDAC inhibitors (HDACi) lead to the temporary but significant reduction in the H3.3K27M protein (up to 80%) in multiple glioma cell lines expressing the H3.3K27M histone variant, despite increasing *H3F3A* mRNA expression. The H3.3K27M occupancy at the chromatin is greatly reduced upon HDACi (SB939) treatment, as shown by ChIPseq analysis. H3.3K27M loss is most striking at SB939-upregulated genes suggesting the role in repression of these genes. In addition, genes previously reported as H3K27M-dependent become downregulated in response to SB939 treatment. We discover that the SB939-mediated loss of H3.3K27M is partially blocked by a lysosomal inhibitor, chloroquine. Moreover, the loss of H3.3K27M is facilitated by co-occurrence of H2A.Z, as evidenced by the knock-down of H2A.Z histone isoforms. ChIPseq analysis confirms the occupancy of H3.3K27M and H2A.Z at the same SB939-inducible genes.

Altogether, we provide new insight into disease-specific mechanism of HDAC inhibition and demonstrate pharmacological modulation of the oncogenic H3.3K27M protein levels. These findings open a new possibility to directly target the H3.3K27M oncohistone, which may be exploited in future therapies.

Abstract No. 63

Exploring calcium dynamics in the astrocyte-medulloblastoma crosstalk via optical biosensor imaging

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Background: Recent evidence highlights the presence of tumor-associated astrocytes alongside non-malignant astrocytes in the microenvironment of medulloblastoma (MB), yet their contribution to tumor development, growth, invasion, and treatment response remains poorly understood. We observed high GFAP expression in SHH MB patients with altered astrocyte morphology, suggesting astrocyte reactivity may evolve alongside tumor progression. Additionally, tumor [Ca2+]i signaling plays a crucial role in cancer progression, but information on its profile in medulloblastoma remains limited. Our study aims at elucidating the regulatory role of astrocyte reactivity on tumor proliferation and tissue invasion within the astrocyte-MB network via tracking calcium dynamics.

Methods: By utilizing optical biosensor imaging, we track the propagation of intracellular calcium signals ([Ca2+]i) in real-time, thereby revealing physiological dynamic interactions between tumor cells and astrocytes. Toward that, we established live GCaMP6-Ca2+ imaging in SHH MB cell lines and ex vivo organotypic cerebellar slice cultures (OCSCs) using fluorescence and two-photon microscopy. We use pharmacological and genetic interference strategies to probe molecular regulators.

Results: SHH MB cell lines expressing GCaMP6 exhibit repetitive [Ca2+]i transients in monoculture and tumor-spheroid implanted OCSCs. We furthermore found that short-term interaction between astrocytes and MB tumor cell spheroids increases cerebellar astrocyte activity, thereby mimicking tumor-associated astrocytic reactivity. We now investigate the impact of gliotransmission and electrical coupling related to astrocyte reactivity on Ca2+ communication through gap junction coupling or GABA paracrine signaling in the tumor microenvironment and determine its functional impact on tumor cell growth and tissue invasion.

Conclusions: This study uncovers a novel crosstalk between MB tumor cells and tissue-resident astrocytes that results in altered astrocyte activity, and it introduces a new tissue model for assessing drugs targeting the tumor-microenvironment interaction.

Abstract No. 64

Cellular and molecular mechanisms at the blood-brain barrier driving melanoma metastasis

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Metastasis of melanoma cells into the brain requires the extravasation of melanoma cells across the blood-brain barrier (BBB). Understanding the cellular and molecular mechanisms of this process is required for a treatment strategy that inhibits melanoma metastasis into the brain. Using primary mouse brain microvascular endothelial cells (pMBMECs) as an in vitro BBB model, we demonstrated an important role of melanoma VLA-4 engaging endothelial VCAM-1 in breaching the BBB. In vitro live cell imaging documented an exclusive junctional pathway of melanoma cell intercalation into the BBB which was associated with a decrease in barrier integrity. Biochemical analysis confirmed melanoma cell mediated degradation of the endothelial cell junction protein PECAM-1. The importance of the BBB junctions to withstand melanoma cell extravasation was confirmed in vivo by a significant increase in melanoma cell extravasation across the BBB in PECAM-1 knockout mice compared to wildtype controls. In vitro, the MMP-inhibitor GM6001 proved successful in protecting the pMBMECs from melanoma cell induced BBB disruption. In summary, our data suggest that protection of the BBB from barrier disruptive treatment and avoidance of inflammatory states are important measures to prevent the development of melanoma metastases in the brain. Furthermore, we propose that a combination of anti-VLA-4 treatment and targeted protease inhibition could reduce the development of brain metastases from VLA-4 positive melanoma.

Abstract No. 65

Automated and Personalized Glioblastoma Tumor Organoid Drug Screening Platform Expose Sensitivity to Proteasome and HDAC Inhibitors

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Personalized drug screening aims to improve the survival of glioblastoma (GBM) patients by identifying effective patient-individual drugs. Therefore, we conducted an automated high-throughput drug screening (aHTS) on standardized glioblastoma tumor organoids (TOs).

Standardized TOs were generated by dissociating fresh GBM tissues into single-cell suspensions mixed with an extracellular matrix. TOs were assessed by light microscopy, IHC, and IF. aHTS was performed using the liquid handler Hamilton MicroLAB STAR and 166 FDA-approved antineoplastic drugs. Human glioma cell lines NCH82 and NCH89 were used for in vitro validation experiments.

GBM TOs fully compacted after two days and the size remained stable over 10 days. TOs proliferated over time as seen as a steady increase in the ATP signal. IF staining for tumor cells (GFAP) and extracellular matrix (Tensascin C) confirmed GBM-tissue-like structures. aHTS was performed on TOs from 11 GBM patients including two recurrent tumors. The most effective drug classes were proteasome inhibitors (carfilzomib, bortezomib, ixazomib), and HDAC inhibitors panobinostat and romidepsin. Potential therapeutic drug levels (Cmax/IC50>1) were only achieved by the proteasome inhibitors and HDACi romidepsin. The drug targets of proteasome inhibitors (PSMB5) and romidepsin (HDAC1/2) were successfully validated by RNAi experiments in glioma cell lines.

Taken together, we established an automated high-throughput drug screening platform for GBM TOs and identified proteasome and HDAC inhibitors as promising drugs.

Abstract No. 66

Exploring the Immune-Mediated Glioma Eradication Potential of Oncolytic Immunovirotherapy

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Oncolytic herpes simplex viruses (oHSVs) have shown promise as effective treatments for various cancers. We previously demonstrated the efficacy of R-115, an oHSV retargeted to human HER2 and expressing murine IL12 (mIL12), in a high-grade, immunocompetent murine glioma model, established through orthotopic transplantation of PDGFB-induced primary gliomas engineered to express HER2.
For the current investigations, we first analyzed the stability of the treatment to drug and schedule variations, that have been shown not to interfere with the efficacy of R-115. Next, recognizing that high-grade gliomas are composed of cells expressing varying levels of specific marker molecules, we examined the treatment performance on a mixed population of tumor cells where only half of them expressed HER2.
Surprisingly, results showed similar overall survival and tumor eradication rates between HER2-pure and -mixed models, with approximately a quarter of animals undergoing complete glioma eradication. Cured mice were rechallenged with purely HER2-negative cells and about 60% of them did not develop gliomas, indicating a robust immune response capable of rejecting secondary transplanted tumors. Plasma analysis revealed increased immunoreactivity against glioma cells in R-115 cured mice, along with elevated levels of immune cell activation markers upon co-culturing R-115 treated mice's splenocytes with both HER2-positive and -negative cells. Moreover, analysis of co-cultured tumor cells showed that splenocytes from cured mice specifically restored MHC-I expression and killed tumor cells.
Altogether, our results highlight R-115 anti-cancer potential resulting in an agnostic vaccination against glioma cells. These effects suggest that mIL12-expressing oHSV could effectively target residual cells post-surgical resection and infiltrating cells distant from the virus administration site.

Abstract No. 67

The therapeutic potential of epigenetic immune remodeling: primary vs secondary brain malignancies

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Introduction: Glioblastoma multiforme (GBM), is the most prevalent and aggressive primary brain malignancy, with a dismal prognosis. Despite therapeutic advancements, GBM remains highly refractory to standard treatment and to immunotherapy with immune-checkpoint inhibitors (ICIs). Noteworthy, melanoma brain metastases (MM-BM), that share the same niche as GBM, frequently respond to current ICI immunotherapies. The potential role of epigenetic modifications in regulating GBM biological processes underscores the possible role of targeting epigenetic drivers as a therapeutic opportunity in GBM. The latter could be used to boost the efficacy of immunotherapy.
Methods: Transcriptome and methylome analyses were performed to study the differences in the constitutive expression profiles of GBM (n=14) vs MM-BM (n=12) cell lines generated from patients' derived tumor biopsies, and to investigate the modulatory effects of the DNA hypomethylating agent (DHA) guadecitabine among the different tumor cells.
Results: The results of the comparative transcriptome/methylome analyses demonstrated an enrichment of biological processes associated with tumor growth, invasion, and extravasation, with the inhibition of MHC class II antigen processing/presentation machinery (APM) and of CD4 T cell proliferation, in GBM vs MM-BM cells. This evidence contributes at least in part to explain the limited success of ICI therapy in GBM compared to MM-BM. Exposure to DHA shaped GBM cells towards a more immunogenic phenotype, close to that of MM-BM. In detail, we observed promoter hypomethylation and consequent up-regulation of genes involved in T and B cell activation, proliferation and migration, as well as in MHC class II antigen processing/presentation and Type I/II/III IFN-mediated signalling.
Conclusions: DHA treatment represents a promising strategy to increase the immunogenicity of GBM cells, closed to that of MM-BM. These results support the rationale to develop new DHA-based immunotherapeutic combinatorial approaches for the treatment of GBM.

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Abstract No. 68

Setting Up a Mouse Neural Organoid Model of Glioma Progression

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Early stages of glioma progression, following the first molecular lesion, are still largely unknown. The use of in vivo murine model to study these aspects pose challenges due to limited access to early stages, struggles in maintaining early-stages tumor cells, and challenges in manipulating complex environments. Mouse neural organoids emerge as an accessible and manipulable in vitro alternative. The aim is to set up an in vitro model of glioma progression using mouse neural organoids. For organoid generation, murine embryonic stem cell line E14Tg2A was seeded under low-attachment culture conditions to form embryoid bodies. These were directed towards neural tissue differentiation under static conditions before transitioning to dynamic culture. At day 30, 55% of analyzed organoids displayed neural rosettes composed of neural progenitors (Sox2+/Nestin+) and radial glia cells (GLAST+). Additionally, all the organoids featured more differentiated neuronal cell types, including early (beta-III tubulin+) and post-mitotic neurons (NeuN+). As for glia cells, astrocytes (GFAP+) were detected in 90% of organoids analyzed. As a proof of concept for tumor generation, mouse high grade glioma primary cells (mHGG) were co-cultured with day 30 brain organoids in static condition for 24 hours. Non-attached mHGG cells were washed and organoids were transferred to agitation. Although the number of mHGG cells does not significantly change over time, after 30 days from the start of the co-culture there is a significant increase in infiltration, measured as the median distance of mHGG cells from the edge of organoids (10 days: 77.2±22.9 micrometer; 20 days 71.2±17.5 micrometer; 30 days: 107.0±26.6 micrometer; 40 days: 99.6±20.3 micrometer; p.value<0.01). Despite inevitable variability between organoid batches, these preliminary results suggest that our mouse neural organoid model supports the maintenance of mHGG cells. Therefore, this model holds promise as a valuable tool for studying the progression of gliomas in a controlled in vitro environment.

Abstract No. 69

Inhibiting Tyrosine Kinases with 2-Styrylquinazoline: A Novel Approach to Glioblastoma and Leukemia

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We examined the molecular mechanisms of action of a 2-styrylquinazoline derivatives, that are known for their high anti-cancer potential, particularly against glioma and leukemia cells harboring different p53 protein mutations. Despite their structural resemblance, compounds within the styrylquinazoline group exhibit a diverse array of action mechanisms, previously only superficially studied in the context of p53 protein reactivation. Our investigations aimed to fill knowledge gaps concerning the pharmacodynamics of this class of drugs, focusing on their effect on the biochemistry of cancerous cells and their interaction with various molecular targets.

Employing IS20, identified as one of the most promising derivatives within our tested group, we assessed its antitumor efficacy alongside its capacity to inhibit specific tyrosine kinases in ABL and SRC family that are known to play pivotal roles in cancer progression and play a significant role in glioblastoma. The research delved into IS20's impact on crucial cellular processes including metabolism, redox homeostasis, signaling pathways, and the induction of cell death mechanisms such as apoptosis and autophagy. Further investigations revealed IS20's broad impact on various proteins implicated in oxidative stress management, iron regulation disrupting the cellular redox balance through a mechanism of iron ion chelation, which induces oxidative stress leading to cell death. These insights into the molecular action of IS20 underscore the compound's potential as a multifaceted tool in cancer therapy, capable of engaging multiple targets within the cancer cell's regulatory framework.

Through biochemical and molecular research on glioblastoma and leukemia cell models, we hope to board the understanding of cellular interactions on tyrosine kinase inhibitors with a styrylquinazoline backbone, providing new avenues for glioma and leukemia treatment.

Keywords: 2-Styrylquinazoline, Glioblastoma, Leukemia, Oxidative Stress, p53 Protein Mutation, Cancer Treatment, Tyrosine Kinase Inhibitors

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Abstract No. 70

Exploring post-surgical microenvironment dynamics and personalized drug delivery strategies to inhibit recurrence in nanomedicine treatments for glioblastoma

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Glioblastoma (GBM) is an incurable brain tumor. Surgery is commonly performed several weeks before chemoradiation, but recurrences inevitably lead to patients' death. Local treatment is a promising approach for GBM, to bypass the blood-brain barrier and achieve high drug concentration in proximity of residual tumor cells. Our previous work showed that the administration of a Lauroyl-gemcitabine lipid nanocapsules (GemC12-LNC) hydrogel in the post-surgical cavity increases the survival of GBM-bearing rodents. The hydrogel is injectable, well tolerated and able to load two active molecules but, despite this, is unable to prevent the onset of recurrences. To increase the efficacy of our local treatment, the addition of a drug able to target the immune cells of the post-surgical microenvironment (SMe) which favours recurrences and treatment resistance, needs to be explored. Here, we aim to define a combinatory regimen of one chemotherapeutic drug and one immunomodulatory drug able to target both tumor and immune cells within the SMe to prevent GBM recurrences. By analysing the SMe via several imaging and analytical techniques (nuclear and biphotonic imaging, multiparametric flow cytometry), we identified therapeutic windows that could be exploited for systemic administration of immunomodulatory drugs, and cellular targets that are overexpressed in the SMe. Based on these results, we have selected SMAC-mimetic small molecule for its pro-apoptotic and immunomodulatory properties. We have analysed it in combination with GemC12-LNC, and this showed a synergistic effect on GL261 murine glioma cells and GBM9 primary human cells (analysis via Synergy Finder software). This combination has also been tested in tumoroids derived from GBM patients, and in vivo on an orthotopic GBM resection model showing a delay in the onset of recurrences.
In conclusion, the characterization of the impact of surgery on GBM recurrences led to the identification of a promising therapeutic combination for the treatment of GBM.

Abstract No. 71

ML-IAP protein at the crossroads between stemness and immunomodulation

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The treatment of glioblastoma (GB) remains a major clinical challenge due to the presence of cancer stem cells and of an immunosuppressive microenvironment driven by tumor-associated macrophages (TAMs). We have previously identified that Melanoma Inhibitor of Apoptosis (ML-IAP) protein, an endogenous caspase inhibitor, is a marker of poor prognosis in patients with GB, overexpressed in glioblastoma stem cells and TAMs. Our study aims to elucidate the specific involvement of ML-IAP protein in tumor stemness and in immunomodulation.
To this end, we used two approaches, one *ex vivo* using human-derived stem-like cells, and one *in vivo* using an immunocompetent mouse model. In both models, ML-IAP expression was down-regulated in cancer cells by shRNA.
In the stem-like cell model, ML-IAP down-regulation significantly decreased proliferation and self-renewal, while promoting differentiation and cell death. By using assembloids, which are co-cultures of mini-brains and stem-like cells, we showed that ML-IAP down-regulation inhibited tumor cell migration and invasion. *In vivo* experiments revealed that orthotopic graft of mice with shML-IAP GL261-GFP cells extended mice survival twofold compared to mice grafted with shCTRL cells. To better understand the role of ML-IAP in tumor-tumor microenvironment crosstalk, we performed transcriptomic analyses by bulk RNA sequencing of shCTRL and shML-IAP GL261-GFP mouse tumors. Preliminary results revealed that the activation of oncogenic pathways as well as the establishment of an immune response is delayed in shML-IAP tumors correlating with mouse survival. A deeper analysis of these results will be instrumental to select pathways and/or genes to antagonize, the final goal being to find a combinatory regimen to eradicate in the long term the tumor. Collaborations for technology transfer are underway, aiming to translate these findings into clinical applications.

Abstract No. 72

Development of an immunocompetent murine model of EGFRvIII-expressing glioblastoma

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Glioblastoma, the most common and aggressive malignant brain tumor, presents limited treatment options and a median survival time of approximately 15 months post-diagnosis.
EGFR coding gene is one of the most frequently altered in glioblastoma, with the EGFRvIII deletion mutation (exons 2-7) being notably prevalent.
EGFRvIII is widely recognized for its involvement in tumor progression and association with poor prognosis. Given its therapeutic significance and the imperative for effective treatments against this tumor type, we developed a preclinical model of EGFRvIII-expressing glioblastoma.
We exploited our established glioblastoma model based on PDGF-B oncogene overexpression in embryonic neural progenitor cells followed by inoculation into the brains of adult mice, resulting in PDGF-B-induced glioblastoma. We then transduced two primary tumor cell cultures from this model with replication-defective retroviruses encoding EGFRvIII and the reporter gene BFP. We enriched EGFRvIII-expressing cells through BFP reporter gene-based cell sorting. The evaluation of EGFRvIII expression over time showed a stable percentage of EGFRvIII+ cells (ranging from 75 to 90%), as confirmed by flow cytometry and immunocytochemistry.
To evaluate their tumorigenic potential, EGFRvIII+ cells were intracranially transplanted into BALB/c immunocompetent mice, resulting in secondary tumor formation. We assessed model stability and potential EGFRvIII expression loss by analyzing cells from EGFRvIII glioblastomas for PDGF-B and EGFRvIII expression, with the secondary tumors retaining EGFRvIII expression levels resembling their parental counterparts.
Furthermore, preliminary evidence indicates that these cells are susceptible to infection by oncolytic viruses targeting the EGFRvIII variant, showing that our model displays a great potential to evaluate targeted therapies against EGFRvIII.
Our preliminary data suggest that the developed model of EGFRvIII-expressing glioblastoma holds promise as a preclinical tool to evaluate EGFRvIII-targeted therapies and address other biological inquiries related to glioblastoma, thereby enhancing our understanding and potentially advancing treatment options for this aggressive brain tumor.

Abstract No. 73

Cancer-associated fibroblasts from lung cancer brain metastases promote cancer cells migration and invasion, but inhibit their growth

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BACKGROUND
Brain metastases (BrM) are a frequent complication of lung cancer with dismal prognosis. The presence of cancer associated fibroblasts (CAFs) was recently described in the BrM microenvironment, but their engagement in BrM biology is largely unknown. In this study, we investigated the effect of CAFs on cancer cells in the brain metastatic microenvironment.

METHODS
BrM-derived CAF (BrM-CAF) and cancer cells (BrM-CC) were isolated from human lung cancer BrM samples. Normal fibroblasts were isolated from subgalear connective tissue. All cell cultures were characterized using immunocytochemistry and fibroblast cultures underwent RNA sequencing. Migration was assessed using a transwell assay. Invasion was evaluated using a 3D spheroid-based assay. Cell growth was quantified using a label-free cell counting kinetic assay.

RESULTS
BrM-CAF expressed canonical fibroblast markers and their fibroblast-like phenotype was verified by RNA sequencing. BrM-CC expressed epithelial markers. Conditioned media from BrM-CAF stimulated the migration of BrM-CC. In the invasion assay, the cancer cell invasive area was bigger in the presence of BrM-CAF; in addition, we observed a different pattern of invasion in BrM-CAF-containing spheroids, with more protrusions being formed. Direct co-culture with BrM-CAF diminished cancer cell proliferation in a dose-dependent manner.

CONCLUSION
Our results demonstrate that BrM-CAF influence cancer cell behavior in the microenvironment of lung cancer brain metastases; BrM-CAF promote cancer cell migration and invasion, but decrease their proliferation. These results underline the importance of further research focused on understanding the importance of BrM-CAFs.

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Abstract No. 74

Towards liquid biopsy for glioblastoma: relying on plasma extracellular vesicles for tumor diagnosis and monitoring

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Glioblastoma (GBM) is the most aggressive adult brain tumor. The achievement of a specific diagnosis for GBM is challenged by the limitations and risks of current standard methods like neuroimaging and solid biopsy. The morbidity associated with tissue sampling and GBM's evolving nature hinder its monitoring by solid biopsy. To address this, we seek circulating biomarkers for non-invasive GBM detection and monitoring, focusing on Extracellular Vesicles (EVs) that cross the blood-brain barrier and whose molecular cargo is specific to the cell of origin.

We isolate EVs using Size Exclusion Chromatography from 2mL of plasma, characterizing them through immunoblot, flow cytometry and transmission electron microscopy. EV concentration and size are measured by Tunable Resistive Pulse Sensing in 50 GBM patients pre-surgery and in controls, including 100 healthy individuals and 50 patients with GBM-mimicking brain malignancies. We monitor changes in EV concentration over time in 44 GBM plasma samples to track GBM evolution. We analyze EV surface proteins using multiplex flow cytometry and the total EV proteome via mass spectrometry, in healthy individuals, and in matched pre- and 72h post-operative GBM to identify GBM-specific biomarkers.

Plasma EVs are more abundant and larger in pre-surgery GBM plasma compared to controls. GBM post-operative EV concentration and size significantly decrease, serving as potential indicators of GBM burden. Multiplex flow cytometry reveals similar expression profiles in GBM and control EVs, suggesting that GBM-derived EVs in circulation are diluted in non-tumor EVs. Yet, we identified the enrichment of CD63, CD81, CD8, and HLA-DRDPDQ on GBM EVs.
Proteomic profiling identifies 117 proteins upregulated in GBM samples, whose ontology recalls pathways of the complement cascade.

In conclusion, assessing plasma EV parameters offers a reliable, non-invasive method to complement neuroimaging, enhancing diagnostic accuracy and guiding personalized treatment strategies for GBM care. Liquid biopsy implementation holds promise in GBM management.

Abstract No. 75

T cell trafficking and metabolic adaption in brain metastasis

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Limited therapeutic efficacy of classical therapies such as surgery, radiotherapy, or novel immunotherapies does not show long-term efficacy, resulting in a median survival of patients with brain metastases (BrM) of only a few months. A better understanding of the unique BrM tumor microenvironment (TME) could lead to improved therapies. Of particular interest is a detailed understanding of the T cell compartment that can be observed in BrM. Here, we aim to gain deeper insights into T cell biology, infiltration, and adaptation to the TME of BrM to overcome treatment resistance. Using a breast-to-brain metastasis model and RNA sequencing approaches, we analyzed transcriptomic profiles of BrM-associated tumor infiltrating lymphocytes and observed an exhausted phenotype that prevents the T cell anti-tumor activity. Changes in several metabolism associated genes and processes were observed in particular in CD8+ T cells. We identified several metabolites which can be targeted and used to improve the immune response to BrM. Furthermore, we investigated in this project the impact of diverse vascular structures on the T cell infiltration to BrM like high-endothelial venules, the glymphatic system or blood vessels. Immune fluorescence staining and spatial transcriptomic data revealed the presence of lymphatic endothelial as well as high-endothelial venules and changes in the polarization of the astroglia system. Taken together, in this project we seek to study the influence of metabolic adaption of the T lymphocyte compartment and infiltration in BrM. First insights confirm the presence of lymphatic structures in the context of BrM as well as a metabolic adaption of infiltrated T cells. Future investigations will analyze and quantify T cell infiltration in relation to the different vascular structures found in the TME of BrM. Furthermore, induced changes in the immune metabolism will reveal the potential of metabolic modulators.

Abstract No. 76

Deciphering the Interplay Between Glioma Progression and Immune System

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In the dynamic interplay between gliomas and the immune system, phenotypic changes occur on both sides during cancer progression, yet the mechanisms remain partially understood. Here we used a glioma model, based on somatic gene transfer of PDGF-B, that recapitulates glioma progression. PDGF-B overexpression results in the formation of low-grade gliomas (LG) that are immunostimulatory and fail to engraft in immunocompetent mice. Later on, these tumors progress into high-grade gliomas (HG) with a pro-tumorigenic M2 infiltrate and are able to generate secondary tumors in immunocompetent hosts. Notably, we showed that LG cells can successfully graft in immunodeficient NOD/SCID mice1.
LG transplants in syngeneic mice depleted for specific immune populations (CD4+, CD8+, NK cells) show that CD4 lymphocytes are crucial for initiating immune surveillance and for recruiting CD8 lymphocytes. Further, RNAseq analysis of mouse splenocytes co-cultured with HG cells highlighted a potent immunosuppressive environment orchestrated by HG cells, marked by downregulation of MHCII and interferon signaling genes. Moreover, flow cytometric analysis on these co-cultures shows that HG cells induce a strong reduction of CD8+ and NK cytotoxic cells and these effects depend on cell-cell interaction but are independent from the presence of CD4+ lymphocytes. Interestingly, our data indicate that HG cells assist in vivo grafting of LG cells, suggesting that the immunosuppressive environment induced by HG cells could tolerate also immunostimulatory LG cells.
These findings underscore the critical role of cytotoxic cells in immunosurveillance, pointing to the dampening of their activity by gliomas as a key strategy for immunoevasion. This evasion mechanism, however, appears to be indirectly influenced by CD4+ lymphocytes, which regulate the recruitment of cytotoxic cells, highlighting a complex interplay between glioma and immune system.

1 Appolloni I et al., Cancer Lett. 2019 - PMID: 30312732

Abstract No. 77

Longitudinal blood plasma proteomics stratifies glioblastoma patients in two groups that differ in overall survival

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Glioblastoma (GBM) is the most malignant brain cancer. Almost inevitably fatal, it has limited treatment options, few clinically useful biomarkers, and short overall survival (OS). Sampling tissue biomarkers for GBM is invasive to the skull, making the blood a non-invasive source of circulating biomarkers.
To discover potentially new blood biomarkers for GBM, we performed in-depth plasma proteomics in two independent GBM patient cohorts by high-resolution isoelectric focusing fractionation (HiRIEF), coupled with a liquid chromatography and mass spectrometry (LC-MS/MS) analysis.
We longitudinally analysed the plasma proteome dynamics in a discovery cohort of 53 GBM patients in samples collected before surgery and at three time points after surgery. Through consensus clustering, based on treatment-naïve plasma protein levels, we discovered that GBM patients can be stratified in two plasma proteome patient groups (PPG). PPG patients had differential plasma levels of GBM-enriched and cancer-signalling proteins. The plasma proteome showed correlation with radiological parameters and survival differences, further manifested in shorter OS in PPG2 patients, after adjusting for age, sex, and treatment in survival analyses. Using supervised machine learning models, we identified panels of proteins that may serve as potential prognostic biomarkers by determining the PPG status. In a longitudinal follow-up, we observed alterations in the plasma proteome of GBM patients on average one month after surgery, whereas the plasma protein levels at later time points reverted to pre-surgery levels. Finally, through HiRIEF LC-MS/MS, we validated the major findings in a validation cohort of 74 patients and analysed the PPG-differentially altered proteins in relation to a group of 36 healthy controls, matched for age and sex distribution.
In summary, based on the blood plasma proteome we discovered two GBM groups that differ in OS, identified panels of plasma proteins that can accurately determine PPG status, and showed that the plasma proteome changes after surgical intervention.

Abstract No. 78

Image-based screening reveals tumor-suppressive effects of the neuroprotective agent prostetin/12k in medulloblastoma.

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Background: Enhanced motility and the invasive behavior of medulloblastoma (MB) influence microenvironment interactions and may promote distant spread. Current drug screening focuses on cytotoxic agents, leaving subtle but potentially therapeutically relevant drug phenotypes associated with motility and invasiveness unrecognized. The serine/threonine kinase MAP4K4 is upregulated in MB and promotes invasiveness. Prostetin/12k is a novel MAP4K inhibitor identified as a motor-neuron-protective agent in amyotrophic lateral sclerosis.

Methods: Using advanced imaging approaches, we investigated prostetin/12k as a tumor-suppressive agent in MB and characterized its effects at the molecular, cellular, and tissue levels. We use cell-based 3D invasion assays, ex vivo tissue models, and quantitative microscopy to assess tumor-restraining activities of prostetin/12k, and machine learning to detect compound phenotypes associated with repressed motility. We use tissue and zebrafish larval models to explore compound effects on cell and tissue viability and anti-invasion efficacy. We determined putative prostetin/12k targets in MB cells using phosphoproteomics.

Results: Prostetin/12k outcompetes existing MAP4K inhibitors in repressing basal and growth-factor-induced matrix invasion with no detectable effects on vertebrate developmental processes. A firm correlation between the prostetin/12k -repressed motile behavior and alterations in the tumor cell actin cytoskeleton was established. Mechanistically, prostetin/12k targets actin dynamics and cell-cell adhesion signaling, which may explain its anti-invasion activity. However, we also observed increased interaction of tumor cells with astrocytes and profound repression of the release and deposition of extracellular vesicles in response to prostetin/12k treatment. Phosphoproteomics identified proteins involved in cell-cell adhesion organization and vesicle trafficking as potential molecular effectors targeted by prostetin/12k.

Conclusion: Prostetin/12k effectively inhibits MB cell invasion and displays no toxicities at effective concentrations. Combined repression of cell invasion and of the release and deposition of EVs by MAP4K inhibition could be a novel therapy approach to restrict MB progression in the cerebellar tissue context.

Abstract No. 79

Single Cell Genomics Identifies Anti-Glioma Phagocytic Immunomodulators

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Human gliomas are classified using isocitrate dehydrogenase (IDH)-status as a prognosticator, but a systematic examination to delineate the influence of genetic differences versus treatment effects on ensuing immunity remains uncharted. Beyond understanding the immunity differences, identification of phagocytic immunomodulators was the primary objective of this study. Herein, we uncover glioma immunity is mediated by 22 distinct cell types using single-cell transcriptomics of 145,000 tumor-associated leukocytes from eighteen IDH-classified primary and recurrent gliomas as a discovery cohort (n = 4-6/ glioma subtype). Furthermore, spectral cytometry evaluations confirmed the occurrence of transcriptionally defined cell types in the tumor microenvironment of the extended validation cohort (n = 9-13/glioma subtype). Specifically, brain resident microglia (MG) significantly decreased with glioma recurrence independent of IDH status. Monocytic derivatives exhibited a gradient infiltrative pattern that correlated with glioma severity, of which macrophages acquired antigen presentation cell (APC)-like phenotype in response to treatment, with a concomitant increase in proportions of CD8+ T lymphocytes. In contrast, IDH-wild type gliomas exhibited increased proportions of proliferative myeloid cells, APC-like MG and cytotoxic CD8+ T cells relative to IDH-mutant gliomas. Besides enunciating the IDH and treatment associated immunity differences, we provide a faithful genomics framework for defining macrophage polarization beyond M1/M2 paradigms such as palmitic-, oleic-acid, and glucocorticoid- responsive polarized states. Additionally, as an advance from widely used leukocyte gene signature matrix (LM22), we provide glioma specific leukocyte signatures termed Glio-TIME-36 (glioma tumor immune microenvironment-36) as a robust reference for deconvolution of brain tumor transcriptomic datasets. Interestingly, our reverse translational, in vivo and ex vivo investigations identified anti-glioma phagocytic immunomodulators such as Triggering receptor expressed on myeloid (TREM-2) + phagocytes that efficiently engulf glioma cells. Thus, our study not only provides the most advanced optics of pan-glioma immune contexture for downstream translational and clinical applications but also identifies tractable anti-glioma phagocytic circuit.

Abstract No. 80

Dissecting the role of CSF2Rb-STAT5 signaling in tumor-associated inflammation in brain metastasis

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Tumor microenvironment (TME)-targeted therapies are emerging as promising treatment options for different cancer types. Tumor-associated macrophages and microglia (TAMs) represent the most abundant non-malignant cell type in brain metastases (BrM) and have been proposed to modulate metastatic colonization and outgrowth. We utilized the colony stimulating factor 1 receptor (CSF1R) inhibitor BLZ945 to target TAMs at different stages of the metastatic cascade and observed anti-tumor responses in preclinical prevention and intervention trials in breast-to-brain metastasis models. However, anti-tumor activity was only transient and adaptive resistance mechanisms prevented long-term therapeutic efficacy. Transcriptional analysis revealed the induction of compensatory CSF2Rb-STAT5 signaling in a subset of TAMs leading to neuroinflammatory gene signatures in association with wound repair responses that fostered tumor recurrence. We employed different strategies to block the CSF2-mediated TAM activation, including CSF2 neutralization, genetic silencing of CSF2Rb and pharmacologic inhibition of STAT5. CSF1R inhibition combined with CSF2 neutralization or CSF2Rb knockout led to a complete loss of the remaining TAM population. However, depletion of the CSF2-activated TAM population resulted in only minor improvement of the therapeutic response. In contrast, combination of CSF1R blockade with STAT5 inhibition led to a restoration of the TAM population together with phenotypic normalization and amelioration of neuronal damage. Importantly, this intervention strategy resulted in sustained tumor control (1). Transcriptomic analyses of TAMs and other BrM-associated cell types in response to CSF1R inhibition compared to CSF1R-CSF2Rb vs CSF1R-STAT5 inhibition will help to identify mechanisms that mediate TAM normalization and will provide evidence for optimized TAM-targeted therapies to further improve therapeutic efficacy and mitigate the risk of neurotoxicity.

Reference

1 Klemm et al Nat Cancer. 2021 Oct;2(10):1086-1101. doi: 10.1038/s43018-021-00254-0.

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Abstract No. 81

Thoroughly characterized glioblastoma patient-derived xenograft (PDX) and matching cell line models for preclinical and translational research

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Glioblastoma (GBM) is the most common malignant brain tumor in adults. Its heterogeneity and infiltrative growth limit the success of current standard of care (SoC) therapy and of new therapeutic approaches. There is a need for models that reflect the tumor's complex biology in order to perform key steps of preclinical drug development - from in vitro screening to orthotopic in vivo approaches - under conditions closely resembling the clinical situation. GBM patient-derived xenograft (PDX) models and PDX-derived cell lines can preserve many tumor specific characteristics and are therefore an essential tool for translational research.

We have established a panel of 26 glioblastoma PDX models subcutaneously, 15 additionally orthotopically, on immunodeficient mice. Of these, 22 have been characterized for drug sensitivity and their molecular profile using transcriptome sequencing. Corresponding cell lines were established from six PDX models. They are analyzed for their individual morphology and growth, drug sensitivity and cancer stem cell marker expression via FACS.

Best in vivo treatment responses in s.c. models were observed for SoC temozolomide (TMZ) and irinotecan, with reduced sensitivity in matching orthotopic models. Molecular characterization identified all models as IDH-wt (R132) with frequent mutations in PARP1, EGFR, TP53, FAT1, and within the PI3K/AKT/mTOR pathway. The expression profiles resemble proposed mesenchymal, proneural and classical GBM molecular subtypes. PDX-derived cell lines showed distinct growth characteristics and CD15/CD133/CD44 expression patterns, with individual sensitivity profiles regarding receptor tyrosine kinase and PI3K inhibition, and an overall high sensitivity towards topotecan.

In vitro and in vivo screenings identified topoisomerase inhibitors irinotecan and topotecan as alternative treatment options in our TMZ-resistant GBM models. Our data demonstrates that the established platform of GBM PDX, complemented by matching PDX-derived cell lines for in vitro screens are valuable tools for drug development.

Abstract No. 82

Effects of Protocadherin gamma C3 knockout on radiation sensitivity of glioblastoma cell lines

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Radiation therapy is routinely used to treat glioblastoma. Radioresistance is one of the clinically observed limitations to therapy. New strategies to combat the radioresistance of glioblastoma cells are urgently needed.
Protocadherins (PCDHs) belong to a large family of cadherin-related molecules and are organized into three large clusters, alpha-, beta- and gamma-PCDHs. PCDHs play a role in cell adhesion, cellular interactions and central nervous system (CNS) development. In the CNS, the expression of is well characterized in neurons, astrocytes, pericytes, in epithelial cells of the choroid plexus and in brain microvascular endothelial cells.
One of protocadherins, PCDHGC3, is overexpressed in glioblastoma and its high expression is associated with longer progression-free survival of patients. To analyze the role of PCDHGC3 in the response of glioblastoma cell lines to radiotherapy, we generated PCDHGC3 Knockout cell lines (PCDHGC3 KO) using three glioblastoma cell clines U343, U138 and U87. Cells were irradiated at doses of 8 Gy or 20 Gy and harvested 30 minutes or 24 hours post irradiation. Signaling pathways activated in response to irradiation were analyzed using Western blot and phospho-specific antibodies.
ATM and ATR kinases are involved in the DNA damage repair pathway in glioblastoma and their activation promotes radioresistance. Statistically significant activation of ATM kinase was visible in all cell lines 30 minutes and 24 hours after irradiation. Similarly, activation of ATR kinase was observed in irradiated cells 24 hours after irradiation. No statistically different levels of phospho-ATM or phosphor-ATR could be observed due to PCDHGC3 KO. Furthermore, differences in the activation of AKT, b-catenin, ERK pathway, or caspase-3 and PARP-1 cleavage were not observed at any time point or treatment.
Although PCDHGC3 is highly expressed in glioblastoma and its knockout affects glioblastoma cell line phenotype, it does not appear to play a role in the response of glioblastoma to irradiation.

Abstract No. 83

CD4 and CD8 T cells independently control medulloblastoma tumor growth and dissemination

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Medulloblastoma, the most common malignant brain tumor in children, is challenging to treat and frequently refractory to conventional therapies. While immunotherapy has shown promising outcomes in various cancer types, its efficacy in treating high-grade brain tumors remains poor. It is thus essential to better understand which immune cell types regulate medulloblastoma growth. Using a medulloblastoma mouse model we showed that immune cell-derived interferon gamma (IFNg) induced MHC class I presentation in otherwise negative tumor cells, facilitating their recognition and killing by CD8 T cells. Mimicking IFNg treatment through ectopic IFNg expression in tumors promoted tumor cell killing and prolonged survival of tumor-bearing mice. These results underscore the potential of IFNg as an adjuvant that could enhance T cell-targeting immunotherapies.
Our study further revealed a critical role for CD4 T cells in limiting tumor growth and metastasis. Common direct killing mechanisms for CD4 T cells as well as the notion that CD4 T cells primarily act as helper cells for tumor-killing CD8 T cells were experimentally excluded. Single-cell RNA sequencing of tumor-infiltrating immune cells showed a significant underrepresentation of B cells, plasma cells and phagocytic macrophages in CD4 T cell-depleted tumors. Indeed, subsequent experiments suggested a critical role for B cells and myeloid cells in anti-tumor immune control.
In summary, we found that CD8 T cells can unexpectedly restrict growth of MHC class I negative medulloblastoma cells by interferon-dependent induction of MHC class I presentation. Intriguingly, CD4 T cells and B cells, traditionally overlooked in brain cancer research, emerged as additional potent players in the anti-tumor immune response. Ongoing experiments aim to delineate the specific role of B cells and myeloid cells in inhibiting tumor growth. Future efforts will focus on stimulation of tumor-reactive T cells and their effector cells to develop novel immunotherapies for medulloblastoma patients.

Abstract No. 84

Mannose receptor targeting dendrimer nanocarriers as an efficient tool to deliver therapeutic siRNA to glioma-associated microglia and macrophages

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Microglia and peripheral monocytes massively infiltrate glioblastoma (GBMs) and become polarized to glioma-associated myeloid cells (GAMs), which promote glioma invasion, immunosuppression and angiogenesis. Due to their critical roles as supporters of glioma progression, GAMs are considered to be a promising therapeutic target. However, genetic manipulation of myeloid cells in diseases using small interfering RNA (siRNA) is hampered by the lack of safe and efficient siRNA delivery methods. Mannose receptor (CD206) expressed by antigen-presenting cells drives activated T cells towards a tolerogenic phenotype and is a biomarker of tumor-associated macrophages. We designed and tested the mannose-decorated amphiphilic dendrimer nanovectors (ManAD) for functional siRNA delivery and gene knockdown in GAMs in orthotopic glioma model in mice. The biodistribution analysis of nanocomplexes with fluorescently labelled siRNA showed superior homing of ManAD to the brain as compared to non-decorated carriers. Administration of arginase targeting siRNA with ManAD led to effective knock-down of the target protein in GAMs. As arginase is a hallmark of immunosuppressive GAMs, it resulted in decreased tumor growth in mice. We propose the ManAD dendrimers as a promising innocuous carrier for therapeutic siRNA delivery which opens up new perspectives in functional genomic studies and therapeutic targeting of myeloid cells in brain tumors.
The study supported by National Centre for Research and Development in Poland and French National Research Agency under the frame of Era-Net EURONANOMED “iNanoGun” project.

Abstract No. 85

Deciphering WNT6-driven mechanisms of aggressiveness and resistance to chemotherapy in human glioblastoma

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Introduction: Glioblastoma is among the deadliest human cancers, for which treatment remains largely ineffective. Thus, it is urgent to identify biomarkers predictive of glioblastoma therapy response, and elucidate their underlying mechanisms, ultimately uncovering novel therapeutic opportunities. Our team has demonstrated that WNT6, a WNT pathway activator, is an oncogenic molecule with prognostic value in glioblastoma. Nevertheless, the molecular mechanisms underlying its effects remain mostly undetermined.
Materials and Methods: The transcriptome of two glioblastoma in vitro models genetically-engineered to express differential levels of WNT6 were defined through RNA-sequencing and bioinformatics tools. We explored the effects of WNT6 expression in the efficacy of the glioblastoma standard chemotherapeutic, Temozolomide, in these WNT6-modulated glioblastoma cell lines, both through in vitro functional and molecular assays, and through in vivo orthotopic experiments. RNA-sequencing results were validated and the correlations of WNT6 with classical mechanisms of Temozolomide resistance were investigated in glioblastoma patients' data. Finally, we studied the effects of WNT6 expression in the clinical outcome of glioblastoma patients treated with Temozolomide.
Results: By RNA-sequencing, we uncovered novel enriched processes and pathways that further define the oncogenic roles of WNT6 in glioblastoma, and hinted for an enrichment of terms related to therapy response, corroborating preliminary data suggesting WNT6 could have a role in Temozolomide response. Importantly, WNT6 expression was associated with decreased Temozolomide effectiveness both in vitro and in vivo. Molecular assays and omics enrichment analyses unraveled possible mechanisms through which WNT6 could be reducing Temozolomide effectiveness, namely through effects in DNA repair mechanisms. Additionally, we identified high WNT6 as an independent prognostic biomarker for a decreased survival of chemotherapy-subjected glioblastoma patients.
Conclusions: This work describes WNT6 effects on chemotherapy response in the context of glioblastoma, and sheds light into possible mechanisms through which this molecule may be operating and therapeutically-targeted in future precision medicine approaches.

Abstract No. 86

Exploiting the Relevance of WNT6 in Extracellular Vesicles of Glioblastoma: Clinical and Molecular Insights

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Introduction: Glioblastoma (GBM) is the most common and lethal primary brain tumor in adults, partly due to technical challenges in diagnosis and monitoring. Liquid-biopsies based on extracellular vesicles (EVs) offer a promising approach to overcome the existing limitations, as EVs are enriched in the blood of GBM patients. Yet, EV-based biomarkers with true clinical value are still lacking. Our team previously linked WNT6, a WNT pathway activator, to increased aggressiveness and poor prognosis in GBM. Particularly, WNT6 has been detected in high levels in GBM cell line-derived EVs. This raises the intriguing hypothesis that WNT6 in blood-derived EVs may also have clinical relevance in GBM, which remains to be investigated.
Materials and Methods: EVs were isolated from GBM and lower-grade glioma patient plasma samples and GBM cell lines genetically manipulated to express differential levels of WNT6 and were characterized according to the guidelines from the International Society for Extracellular Vesicles . WNT6 protein and mRNA expression in EVs and tumors was assessed through western blot and qPCR, respectively. RNA-sequencing from WNT6+/- GBM cells and TCGA data from GBM patients was used to assess associations between WNT6 and EV biogenesis.
Results: WNT6 cell-transcriptomes revealed enrichment for several EV- and signaling-related processes, and TCGA data from GBM patients showed statistically significant correlations between WNT6 and EV biogenesis genes. A higher EV concentration was linked with increased WNT6 expression in both cell line-derived EVs and patient EVs. Curiously, WNT6-high GBM patients presented a significantly higher level of plasma EVs than those with WNT6-low GBMs. Additionally, WNT6 expression levels in EVs were positively correlated with WNT6 expression in the respective glioma tumor.
Conclusion: WNT6 expression in GBM cells is associated with EV biogenesis. Moreover, WNT6 levels in GBM patients' blood-derived EVs mirror its tumoral expression, highlighting its potential application for liquid-biopsy approaches in GBM.

Abstract No. 87

Development of arrays for the application of Tumor Treating Fields (TTFields) to brain tumors in mouse models

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OBJECTIVE: Tumor Treating Fields (TTFields) therapy, an approved treatment for WHO grade 4 gliomas, is delivered non-invasively via arrays on the skin surrounding the tumor. Gliomas are primarily studied in mouse models in vivo, but due to the lack of suitable arrays for TTFields application to the mouse head, research in this area is constrained. Our goal was to develop arrays tailored to the small dimensions and unique geometry of the mouse head."

METHODS: Different array layouts were tested to identify one that optimally accommodates for the mouse head's geometry while minimizing head movement restriction. Different adhesive tapes were tested for attaching the arrays to the skin to ensure good adherence and thus provide efficient treatment delivery. Measurements of the electric field intensity were carried out to validate that the selected array layout delivers sufficient field intensity to the target region.

RESULTS: To address the small mouse head size, we subdivided the head arrays into two smaller disks and the opposing arrays are placed on the torso. In addition, we identified a thin, transparent adhesive tape that has adequate adhesiveness, can be easily removed without leaving adhesive residue on the skin, and facilitates correct positioning of the arrays on the head of the mouse. Field measurements showed an intensity ≥1 V/cm root mean square and a current ≥50 mA, and the arrays successfully applied TTFields to mice with an application time of ≥75%, fulfilling the requirements for effective tumor treatment.

CONCLUSION: Our newly developed arrays for the treatment of head tumors in mouse models enable efficient delivery of TTFields in vivo via a flexible construct that adheres strongly to the skin. By facilitating the delivery of TTFields to the heads of mice, we will expand the scope of brain tumor treatment research and will contribute to further advance this field.

Abstract No. 88

BRAFV600E mutation and PTEN deletion in neural stem precursor cells give rise to glioma and neurofibromatosis

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The RAS/BRAF/MEK/ERK pathway is highly mutated in cancer, with mutations of the BRAF gene accounting for about 7% of cancers. The most frequently observed BRAF mutation is the V600E, which induces persistent activation of BRAF, inhibiting its inactivation and leading to a continuous pathway stimulation.
In the nervous system, BRAFV600E mutation has been found among low-grade glioma, pediatric oligodendroglioma-like tumors, pediatric glioblastoma, and adult epithelioid glioblastoma, as well as in peripheral nervous system tumors.
To understand if BRAFV600E mutation can drive nervous system tumor formation, we developed a mouse model in which BRAFV600E, along with Pten mutations, are driven by the Tamoxifen-inducible Sox2-CreER, a deleter specifically active in Neural Stem/Progenitor Cells (NSPC). We observed that Pten deleted and BRafV600E mutated central NSPCs are prone to transform into low-grade gliomas, while peripheral NSPCs transform into paraspinal plexiform neurofibromas and MPNSTs. To prove that NSCPs were the tumor cells of origin, we specifically deleted Sox2 in these cells by crossing BRaf/Pten mice with conditional Sox2loxP/loxP mice. None of the Sox2-deleted BRaf/Pten mice developed tumors compared to Sox2-wildtype BRaf/Pten mice.
In vitro analysis on BRaf/Pten mutated NSPCs, revealed that these cells show increased proliferation and preferentially differentiate towards oligogendroglial-like fate.
Moreover, BRaf mutated NSPCs show increased Sox2 protein levels compared to wildtype. RNA-seq and transcript analysis revealed that Sox2 mRNA levels, instead, are equal between mutated and wildtype NSPCs, suggesting that Sox2 increased protein levels are due to its stabilization in the BRaf mutated genotype. In support of this observation, in BRaf mutated NSPCs, Sox2 protein levels do not change following proteasome degradation and translation inhibition. In addition, we observed a biochemical interaction between Sox2 and BRafV600E, suggesting that BRafV600E mutation might be responsible for Sox2 protein stabilization, and this could be the mechanism through which BRaf mutate NSPCs drive tumor formation.

Abstract No. 89

Microenvironmental Influences on Leptomeningeal Metastasis

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Brain Metastases affects up to 10% of patients with solid cancers, and the incidence of leptomeningeal brain metastases (LM) is further on the rise, likely due to improved survival rates among patients with metastatic disease and better treatment options of parenchymal brain metastases. LM prognosis is dismal with a survival of weeks without LM-directed therapy and 2 to 10 months under therapy. This underscores a significant clinical need for innovative therapies targeting LM. Age at diagnosis is a known negative prognostic factor for LM, prompting our investigation into the impact of aging on LM development and progression.

In this study, we utilized intravital microscopy and various preclinical models of LM to provide evidence that the aging microenvironment contributes to LMD development. Specifically, we observed a higher incidence of LM, increased tumor burden, and elevated signs of tumor-associated hydrocephalus in aged mice. Importantly, this phenotype was found to be independent of the immune system. Analysis of the aging microenvironment through proteomic and transcriptomic approaches revealed notable differences in endothelial cells. Our ongoing research is focused on elucidating the downstream pathways involved in increased metastatic growth and developing targeted therapies aimed at endothelial cells to prevent and treat LMD.

Abstract No. 90

Functional and molecular investigations of IDH1-mutant astrocytoma grade 4

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Diffuse glioma is the most common type of malignancy originating from the central nervous system, carrying a dismal prognosis despite standard treatment. A proportion of patients harbor mutations in the isocitrate dehydrogenase 1 (IDH1) enzyme, resulting in production of the oncometabolite 2-hydroxyglutarate. Albeit initial favorable prognosis compared to glioblastoma patients, IDH-mutant astrocytoma grade 4 patients have equally poor prognosis at recurrence. Our group has identified loss of IDH1-mutation in patient-derived cell cultures (PDCCs) established from IDH-mutant tumors, which correlated with increased malignancy. Findings of IDH-mutant and IDH-non-mutant tumor cell populations in tumor tissue of a cohort of 22 IDH-mutant glioma patients proposed that these cells were present already at diagnosis.
To understand the significance of the IDH-mutant and IDH-non-mutant tumor cells in IDH-mutant astrocytoma grade 4 we have performed functional analyses of IDH1-mutant PDCCs at early and late passage and phenotypic analyses of patient tumor tissue. Early passage mutant cultures did not exhibit in vivo tumorigenicity, while tumor formation could be observed in one late passage non-mutant culture. This was linked with increased invasiveness, self-renewal and expression of stemness-markers. Overall, the project provides models of and insight into IDH1-mutant astrocytoma tumor progression.

Abstract No. 91

Molecular and Spatial Insights into Treatment Failure in Pediatric Low-Grade Gliomas

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Background: Pediatric Low-Grade Gliomas (PLGG) are the most common solid tumors in children and young adults, characterised by their slow growth, chronic clinical course, and high treatment burden. While gross surgical resection can be curative, it is often not possible and chemotherapy, targeted therapy, and/or radiotherapy are required for tumor control. However, progression is common, and the reasons for treatment failure remain poorly understood. In our study we seek to define the genetic changes, cellular heterogeneity, its spatial distribution, as well as cell-to-cell interactions and their contribution to therapy evasion and PLGG progression.

Methods: Retrospective clinical data was extracted from the medical records, of patients treated for PLGG at the University Children's Hospital Zurich from 1990 until 2023. Molecular profiling was performed using Foundation One CDx panels. Spatial proteomics profiling will be completed using imaging mass cytometry (IMC).

Results: Ninety-eight PLGG patients with full clinical profile and tumor sample for molecular testing were included. 51% of patients had tumor progression, with a 48% of a the 5-year PFS. Tumors located along midline structures developed progression at higher rates. Earlier age of diagnosis was correlated worse prognosis. 48% of patients had an alteration at the level of BRAF (67% BRAF fusion, 33% BRAF V600E). The presence of co-mutations was detected in 18 patients from our cohort. Analysis of a sub cohort of 33 matched samples from 15 patients pre/post-treatment revealed in some a variation in their genetic markers.

Conclusion: PLGG progression is associated with age of diagnosis and tumor location. Our results thus far suggest that some PLGG tumors harbor more than one alteration in oncogenes, which may dictate a more aggressive clinical course. In depth analyses are ongoing focusing on matched pre-/post-treatment samples, to gain insights of the tumor micro-environment through spatial biology.

Abstract No. 92

Lavender angustifolia essential oil and its terpenic components impair cell proliferation and migration in a cell model of glioblastoma

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Glioblastoma multiforme (GBM) is the most aggressive form of glioma and it is characterized by a highly proliferative and infiltrative behaviour. Due to its aggressive nature and the limitations of current therapies, GBM presents significant therapeutic challenges. One of the goals of the current cancer research is to identify bioactive molecules able to enhance the effectiveness of traditional treatments, and to improve patient outcomes. Promising results have been reported on the ability of lavender essential oil (LO) or its components to inhibit cancer cell growth in some in vitro cancer models, such as colon and prostate cancer, among others. Our investigation was aimed at verifying whether LO and its monoterpenes components could influence tumorigenesis in GBM cell models. We found a significant inhibition of cell proliferation in a time- and dose-dependent manner after LO treatment alone or in combination with Temozolomide (TMZ). Moreover, we observed that LO impairs the migration capabilities of GBM cells, in trans well-based chemotaxis experiments. Following the chemical characterization of LO by GC-MS, we analysed the effects of the most represented terpenes on GBM proliferation. In detail, the proliferation rate of cells was analysed following treatment with Linalool, Borneol and Terpinen-4-ol. All these terpenes, although at different rates were able to induce an impairment of cell proliferation. Ongoing experiments will show if these compounds are also able to mimic the anti-migratory effect of LO. An investigation of the putative molecular mechanisms involved in terpenes effects on cell proliferation and migration is currently ongoing. The research project offers valuable insights into the potential use of terpenes as main bioactive components of LO against GBM aggressiveness. Our study revealed the first approach to assign to terpenes-enriched LO a potential role as a natural adjuvant for GBM therapy.

Abstract No. 93

Spontaneous Cancer Cell Intrinsic Calcium Oscillations drive Brain Metastasis Progression

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Recently, we have shown that primary brain tumours form highly organized, small-world scale-free networks communicating intercellularly via calcium oscillations, reminiscent of neural networks. Such cancer-cell intrinsic neural mechanisms are becoming an increasing focus in the field of Cancer Neuroscience, but it has not yet been shown whether tumours of non-CNS origin also have the ability to form such multicellular networks which sustain proliferative signalling and increase resistance to therapy.

Employing longitudinal intravital two-photon microscopy in awake mice, we demonstrate that brain metastases of melanoma, breast and lung cancer exhibit neural-like spontaneous calcium oscillations in vivo. These oscillations are tightly regulated by an interplay of calcium-induced calcium release and store-operated calcium entry pathways and drive proliferation. Mathematical modelling of these calcium oscillations revealed local interactions between neighbouring tumour cells. Indeed, dye transfer experiments confirmed coupling of brain metastases cell in vitro. Investigating the mechanism of this intercellular communication, we found an upregulation of gap junctions in brain metastases. Indeed, inhibition of gap junctions with three different pharmacological agents significantly reduced calcium oscillations in vitro and led to reduced tumour proliferation both in vitro and in vivo. RNA-Sequencing and phospho-proteomics revealed pro-tumorigenic signalling via CREB. These findings indicate novel parallels between primary and secondary brain tumours. Our future focus is on further unravelling the downstream pathways activated by these calcium oscillations to identify actionable targets, new treatments and enhance our understanding of brain metastases biology.

Abstract No. 94

The role of tryptophan-catabolizing enzymes in immunometabolism in glioblastoma.

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Glioblastoma is the most common and aggressive primary brain cancer in adults. Therapy resistance is one reason for the poor prognosis of glioblastoma, to which immunosuppressive mechanisms in the tumor microenvironment (TME) contribute relevantly. Metabolic processes play a critical role in the interaction between tumor cells and immune cells in the TME. We focus on the role of tryptophan (Trp)-catabolizing enzymes (TCEs) in glioblastoma. TCEs produce metabolites that are able to activate the transcription factor aryl hydrocarbon receptor (AHR) and promote immunosuppression and tumor progression. Our special interest lies in the regulation of TCEs in tumor cells and in different immune cell subsets in glioblastoma. In addition, we study the metabolites produced by these TCEs and their effects in glioblastoma biology. A better understanding of Trp metabolism in glioblastoma is important to better stratify patients with glioblastoma to novel emerging therapies targeting TCEs or AHR in the future.

Abstract No. 95

Putative role of the non-receptor tyrosine kinase FGR in medulloblastoma

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Medulloblastoma (MB) is one of the most common malignant pediatric brain tumors, that exhibit varying developmental origins and distinct biological features. Traditionally, driver genes have mostly been identified by focusing on coding regions of the genome. In a proof-of-concept study, we used conservation scores to discover non-coding constraint mutations (NCCMs) in glioblastoma. We since expanded the analysis to identify and validate genes with increased NCCM burden, and potential function in MB. Using WGS data sets from tumor/normal pairs we intersected mutations with evolutionary constraint to identify NCCM-enriched genes, mutations likely to be regulatory in nature. Intriguingly, several NCCMs in MB are associated with different age of onset.
In adult MB patients, NCCMs occur in, e.g. the gene for FGR, a SRC family kinase. In this locus, we found six NCCMs that increased FGR expression. FGR has been associated with several cancer types but to our knowledge, has not been implied in brain tumors. Our preliminary data now shows altered levels of FGR in MB cells that were CRISPR/Cas9-edited to contain NCCMs. This could explain the increased proliferation and enhanced responsiveness to the SRC kinase inhibitor Dasatinib, we have reported. We have established FGR-overexpressing (FGR-OE) MB cells to study the role of FGR in MB tumorigenesis. Preliminary data indicate that overexpression of FGR results in increased cell proliferation. Inhibiting FGR-autophosphorylation by treatment with TL-0259 decreases proliferation. Further aims of this project are to explore how FGR contributes to MB, its relation to other SFKs (Fyn and Lyn) and potential dependency on SRC activity. As it appears that the NCCMs in the FGR gene have functional consequences for tumor cell phenotypes, we will test the effect of FGR perturbation on MB cell lines representative of different subgroups. The overall goal is to connect non-coding DNA mutations in MB with novel therapy approaches.

Abstract No. 96

Repurposing of Antipsychotic Trifluoperazine for Inhibiting Mitochondrial Function in Diffuse Midline Glioma

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Background
Diffuse Midline Gliomas (DMGs) present significant challenges in neuro-oncology, urging the exploration of novel therapeutic strategies. Repurposing approved drugs, particularly those with diverse effects, holds promise in effectively addressing these challenges. We investigated the potential of trifluoperazine (TFP), an antipsychotic medication and dual DRD2 and calmodulin antagonist, focusing on its mitochondrial-targeted actions in DMGs. DRD2 signaling drives glioma proliferation, while intracellular Ca2+ regulates cellular processes like gene expression, motility, and survival. TFP inhibits DRD2 and calmodulin, inducing irreversible Ca2+ release from intracellular stores triggering the integrated stress response (ISR) pathway. Disruption of calcium homeostasis leads to mitochondrial damage and inhibits cell proliferation, invasion, and survival of gliomas.
Methods
TFP showed robust in vitro anticancer activity against DMGs. We investigated the molecular mechanisms underlying TFP's interaction with mitochondria, emphasizing its effect on mitochondrial function and metabolism. Patient-derived DMG cells were treated with increasing doses of TFP, and cytotoxicity was assessed. Various assays were performed to evaluate mitochondrial morphology, ROS production, mitochondrial membrane potential, calcium levels, ATP levels, metabolic response, migration, and invasiveness.
Results
DMG cells showed increased sensitivity to TFP compared to healthy astrocyte cells. TFP reduced DMG cell metastatic potential, viability, and induced cell death, accompanied by decreased ATP levels and OXPHOS metabolism. DMG cells showed metabolic stress and mitochondrial dysfunction, characterized by decreased membrane potential, increased ROS and calcium levels, and mitochondrial network fragmentation. Further investigations including metabolomics, transcriptomics, proteomics, drug combination assays, and in vivo studies are ongoing.
Conclusions
Our findings suggest a promising repurposing opportunity for psychotropic drugs with established pharmacologic and safety profiles like TFP in treating DMGs, highlighting the importance of mitochondria as a therapeutic target. Further exploration through multi-omics and in vivo studies holds promise for elucidating TFP's full therapeutic potential and its integration into clinical practice.

Abstract No. 97

Nifuroxazide induces G1 arrest and cell death via perturbing centrosome integrity in patient-derived glioblastoma stem cells

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Glioblastoma is a most aggressive brain tumor that constitutes self-renewing and highly tumorigenic glioblastoma stem cells (GSCs). GSCs contribute to poor responses to therapeutics and recurrence. Thus, identifying GSC vulnerabilities is critical, which may provide previously unexplored therapeutic targets for treating glioblastoma. Centrosomes are essential for accurate cell division and viability, whose numerical amplification is a hallmark of several cancer types, offering an attractive intervening point. Here, we reveal that patient-derived GSCs, do not display aberrant centrosome numbers. However, when we genetically abolished CPAP-SIL interaction, bonafide centrosome duplication factors upregulated in GSCs, GSCs underwent G1 arrest, eventually leading to cell death. Intervening CPAP-SIL interaction significantly induced centrosome fragmentation and centrosome loss in GSCs. Furthermore, applying biochemical compound screening, we identified Nifuroxazide as a potent inhibitor of CPAP-SIL interaction which also caused G1 arrest and cell death. Nifuroxazide also significantly perturbed 3D GSC spheroids growth and GSC invasion in human brain organoids and mouse xenografts. These findings underscore the importance of targeting centrosomes in GSCs, although they do not exhibit amplified centrosomes.

Abstract No. 98

Continuation of hypoxia gene programs in post-hypoxic glioblastoma cells

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Glioblastoma (GBM) is the most lethal malignant brain tumor that invariably recurs after standard treatment. Areas of pseudopalisading necrosis, which contain hypoxic and necrotic cells, are a pathological hallmark of GBM. In our recent study, we engineered murine GBM cell lines with a lentiviral HIF hypoxia reporter, HRE-UnaG, to study the relationship between tumor hypoxia and immunosuppression (Sattiraju et al., 2023, Immunity). We observed spatial patterning of tumor-associated macrophages (TAM) and cytotoxic T lymphocytes (CTL) in hypoxic niches during GBM progression. TAM and CTL that had been sequestered in hypoxic zones were reprogrammed toward an immunosuppressive state, while hypoxic GBM cells were negatively enriched for pro-inflammatory pathways. To investigate if GBM cells conditioned by hypoxia during tumor progression retain adaptations in the form of a “hypoxia memory” that favors immunosuppression, treatment resistance and recurrence, we engineered murine GBM cells with a hypoxia induced fluorescent lineage tracing system, consisting of a hypoxia-induced and tamoxifen controlled lentiviral HRE-CreERT2 and knock-in of a lox-stop-lox-tdTomato reporter into the Rosa26 locus. Engineered GBM cells showed faithful induction of tdTomato in vitro at 1% O2 (hypoxia). In mice carrying intracranial tumor transplants, we observed upon tamoxifen administration abundant post-hypoxic GBM cells (positive for tdTomato, negative for HIF target gene GLUT1) with mesenchymal morphology at early tumor stages. At later stages, with tumors forming abundant pseudopalisading necrosis, hypoxic GBM cells were confined to this niche. Post-hypoxic GBM cells, in contrast, were spread widely in vascular-rich tumor regions and showed a morphology and spatial distribution distinct from hypoxic GBM cells. Single-cell RNA-seq studies are currently underway to characterize the transcriptional profile of hypoxic and post-hypoxic GBM cells. Our findings can be leveraged to develop new therapies that can counter hypoxic adaptations in GBM.

Abstract No. 99

Deciphering Clinical Imaging Signatures of Brain Tumors: A Voxel-by-Voxel Correlative In Vivo Two-Photon Microscopy and MRI Platform

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Glioblastoma, the most common primary malignant brain tumor, is characterized by its invasive growth and whole brain colonization and suffers from inadequate detection of the infiltration zone by conventional MRI. Here, we introduce a correlative technology platform to overlay voxel-by-voxel in vivo two-photon microscopy with MR imaging.

Combining patient-derived brain tumor models with in vivo two-photon microscopy, allowed us to follow brain tumor growth with subcellular imaging resolution over time, serving as ground truth for further multimodal MR imaging.
A voxel-by-voxel elastic registration enabled the longitudinal registration between the two imaging modalities, allowing a precise correlation across scales. Furthermore, we establish a framework for adapting these imaging signatures to human MRI through signal level normalization and resolution adaptation between different magnetic field strengths.
Using this technology platform in patient-derived glioblastoma, we uncovered the minimum cell density when standard-of-care T2-weighted MR imaging became positive, enabling the detection of glioblastoma. Interestingly, this correlative workflow allowed us to define novel biological signatures of micrometastatic growth of breast cancers enabling further clinical-translational investigation.
Lastly, we demonstrated how the signal distributions of multimodal MR imaging combined with the microscopic ground truth and deep learning could be used to define clinically relevant imaging signatures of brain tumor growth.

Together, our work opens new avenues for high-resolution, longitudinal studies and offers a robust tool for investigating early disease detection and monitoring.

Abstract No. 100

Patient-Derived Glioblastoma Explants as a Model for the Tumor Immune Microenvironment

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Objective: Even though glioblastoma's resistance to immune checkpoint blockade is known, its underlying mechanisms remain insufficiently explained. This can be in part explained by the lack of models that accurately recapitulate the complex biology of glioblastoma and its tumor immune microenvironment (TiME). In this work, we assessed whether the local TiME is preserved in patient-derived explants (PDEs) and whether PDEs can serve as a suitable model for gaining further insight into glioblastoma's resistance mechanisms.

Methods: PDEs were generated from resected specimens of 8 glioblastoma patients and 3 patients undergoing temporal lobe epilepsy surgery, which serve as our control group. The tumor and immune cell compartments of the PDEs were characterized at baseline and after four days in culture. Tissue quality was assessed using hematoxylin & eosin staining and tissue viability was evaluated using TUNEL staining. The tissue composition and immune landscape were analyzed using immunohistochemistry and imaging mass cytometry (IMC) of the tumors and corresponding PDEs of selected patients.

Results: No significant changes to tissue quality or excessive apoptosis were observed in cultured PDEs. Based on the marker expression quantified by immunohistochemistry, we found that the difference in CD3-positive cells and CD8-positive cells throughout the culture period was statistically insignificant ($p \sim 0.125$ and $p \sim 0.265$, respectively). Furthermore, using IMC, we identified various host cell populations present in the TiME, including astrocytes, different macrophage subpopulations with distinct modes of activation, microglia and other myeloid cells, and their spatial relationship within the TiME.

Conclusion: PDEs preserve histological and immunological characteristics of the TiME over a short time in ex vivo culture. We plan to further evaluate the model's suitability for studying glioblastoma's resistance mechanisms against immune checkpoint inhibitors (ICIs) through testing the changes to the local TiME following in vivo and ex vivo treatment with immunomodulatory drugs such as dexamethasone and ICIs.

Supported by SciSerTec
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Abstract No. 101

Molecular and functional characterization of astrocyte-glioblastoma brain tumor networks

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It is becoming increasingly clear that multicellular tumor networks are one hallmark of yet incurable brain tumors called glioblastoma. We have more recently described gap junction-coupled connections between astrocytes and glioblastoma while their molecular, functional and relevance for tumor biology remained elusive.
Here, using a combination of longitudinal in vivo two-photon microscopy, calcium imaging of astrocyte-glioblastoma communication and correlative single-cell RNA sequencing in comination with a functional imaging dye we aimed to decipher the role of astrocyte-glioblastoma networks for glioblastoma biology.
We found that glioblastoma integration into gap junction-coupled astrocytic networks increased calcium event duration and area while astrocyte remained structurally intact over several weeks after tumor infiltration and regardless of radiotherapy. Interestingly, tumor cells are functionally embedded in astrocytic networks as demonstrated by bidirectional multicellular calcium events of astrocytes and glioblastoma cells. Lastly, our molecular characterization of astrocyte-connected tumor and astrocytes revealed unique ligand-receptor interactions that could serve as therapeutic targets to disconnect astrocyte-tumor networks and tackle glioblastoma.
Together, we revealed how glioblastoma structurally and functionally integrated into astrocytic networks, revealing novel molecular axes of communication that requires further investigation.

Abstract No. 102

Unravelling the role of primary cilium in glioblastoma pathogenesis: Constructing predictive models from patient-derived tumoroids and multi-omics analysis

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Glioblastoma multiforme (GBM) presents a significant challenge in oncology, characterized by its aggressive nature and limited treatment options, resulting in a median survival of merely 15 months despite current therapeutic interventions. GBM's complexity arises from its high plasticity, cellular heterogeneity, and limited accessibility for research. Patient-derived tumoroids offer a promising avenue for investigating GBM, as they faithfully preserve the cellular heterogeneity and patient-specific characteristics of the disease.
The primary cilium has emerged as a focal point in GBM research, acting as both a vital cellular antenna and a central hub for various signaling pathways, including Hedgehog, Wnt, Notch, and TGF-beta. It is believed to exert a significant influence on the glioblastoma stem cell population, characterized by high plasticity and diffusion, which complicates therapeutic strategies.
In GBM, primary cilia have been observed to be rare, absent, or abnormal, yet evidence regarding the consequences on cilium-dependent pathways remains limited. To address this gap, we adopted a pragmatic approach involving the analysis of data from a large cohort. By integrating immunofluorescence-based assessment of cilium dynamics with multi-omics data obtained from patient-derived tumoroids, we aimed to comprehensively explore the relationship between cilium defects and pathway dysregulation in GBM. This comprehensive strategy facilitates an unbiased assessment of the intricate interplay between cilium function and dependent pathways, offering valuable insights into GBM pathogenesis and identifying potential therapeutic targets.

Abstract No. 103

Deciphering immune modulatory effects of ionizing radiation and immune checkpoint blockade in experimental brain metastasis to develop strategies for improved radio-immunotherapy.

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Brain metastasis (BrM) represent a clinical issue with limited effective therapies for patients resulting in miserable patient survival. Even multimodality intervention strategies show no long-term efficacy. This poor patient prognosis emphasizes the urgent need for effective treatment options. Of interest is a detailed understanding of the brain tumor microenvironment (TME), particularly immune cell infiltration and a mechanistic insight in T-cell biology. Therefore, TME-targeted therapies, such as immune checkpoint inhibition (ICI) and different radiotherapy regimens are emerging to interfere with immune suppressive signals by tumor and myeloid cells. Using RNA sequencing approaches, we analyzed transcriptomic profiles of tumor infiltrating lymphocytes (TILs) and observed an exhausted phenotype that prevents T-cell anti-tumor activity. We demonstrated improved survival in response to radio-immunotherapy applied as whole-brain-radiotherapy (WBRT) with increased TIL-recruitment. We investigated effects of WBRT and stereotactic radiosurgery (SRS) on BrM progression and immune cell infiltration. Likewise, efficacy of different ICI with and without radiotherapy was analyzed. We found that ICI therapy with αCTLA-4 combined with WBRT significantly improved OS of mice. Immunophenotyping by FACS analyses revealed no differences in immune cell infiltration in WBRT+αCTLA4 treated mice compared to WBRT. Interestingly, WBRT combined with αPD-1 and αCTLA-4 did not further improve OS. Moreover, SRS with αPD-1 results in significantly improved survival and further enhancement of T-cell infiltration. Summarized, our data demonstrate that alteration of the TME by radiotherapy in combination with ICI could alter T-cell-

mediated anti-tumor activity and modulates the immune suppressive brain TME towards a more favorable milieu for ICI. Further combinatorial in vivo trials, RNA-sequencing as well as spatial transcriptomic analyses will shed light on individual T-cell subgroups and activation states. We believe that such insight will provide scientific rationale for improved radio-immunotherapy treatment schedules to overcome therapy resistance, mitigate the risk of neurotoxicity and improve the therapeutic efficacy for brain metastasis patients.

Abstract No. 104

A strategy for multimodal integration of transcriptomics, proteomics, and radiomics data for the prediction of recurrence in patients with IDH-mutant gliomas

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Isocitrate dehydrogenase-mutant gliomas are lethal brain cancers that impair quality of life in young adults. Although less aggressive than glioblastomas, IDH-mutant gliomas invariably progress to incurable disease with unpredictable recurrence. A better classification of patient risk of recurrence is needed. Here, we describe a multimodal analytical pipeline integrating imaging, transcriptomic, and proteomic profiles using machine learning to 1) describe the biological characteristics of IDH-mutant glioma subtypes categorized by positron emission tomography (PET) and histology, 2) reinforce the integration of positron emission tomography (PET) metrics in the classification of IDH-mutant gliomas, and 3) improve patient stratification with novel signatures of patient risk of recurrence based on gene expression, protein level, and imaging. We provide herein a useful road-map for the stratification of IDH-mutant glioma patients and their risk of recurrence, which will lead to better monitoring of the clinical evolution of the disease.

Abstract No. 105

Towards improving synthetic reporters for phenotypic mapping of glioblastoma states by LSD+

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Cell types and cell states are fundamental units in tissues. In tumors, diverse cell states contribute to intratumor heterogeneity, yet their functional characterization and biological significance remain limited. To address this, we previously developed LSD (Logical Design of Synthetic cis-regulatory DNA), an algorithm for automatically designing synthetic reporters that capture complex cell identities and states. Building on this foundation, we introduce LSD+, which enhances the design of such reporters. LSD+ improves upon the original LSD algorithm by utilizing non-redundant, multi-source large motif collections. This approach not only potentially mitigates experimental biases in motif calling but also more effectively captures consensus motif occurrences. Using the mesenchymal GBM phenotype as a benchmark, LSD+ computationally outperforms previous methods, including the original LSD and manual assembly. These enhancements underscore LSD+'s ability to refine cis-regulatory element selection by modeling motif heterogeneity across extensive collections. Additionally, integrating various transcription factors' modes of action—such as activators and repressors—enables a more effective exploration of complex regulatory syntax. In conclusion, LSD+ not only advances synthetic reporter design but also enhances its scalability, improving the functional characterization of complex cell identities and states.

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Abstract No. 106

EccDNA detected from WES data identifies potential targets for treating glioblastoma

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Although coding regions represent only 2% of the genome, approximately 90% of disease-causing variants are mapped there. Taking advantage of the cost effectiveness of WES data compared to WGS data, we applied our DifCir method for differential analysis of extrachromosomal circular DNA (eccDNA) data to map and detect differentially produced eccDNA/tandem segmental duplication in the chromosome (DPpGCs), in 5 glioblastoma (GBM) tumour samples compared to control blood samples. For fold change 2 in log2 scale and significance level of 0.01, we found 32 eccDNAs up-DPpGCs in tumours compared to blood controls. The statistically most significantly up-regulated eccDNA per gene, DPpGC, was LHFPL tetraspan subfamily member 3, *LHFPL3* (7q22), with one eccDNA mapped from each of the tumour samples. LHFPL3 is reported to be highly expressed in malignant gliomas, and it has been shown that miR-218-5p targets LHFPL3 mRNA and plays a role in preventing the invasiveness of glioma cells; in addition, LHFPL3 is associated with the common chromosomal fragile site FRA7F. We found that there are 6 genomic loci that produce at least 2 up-DPpGCs. We found an increased number of up-DPpGCs on the sex chromosomes, one of which, interleukin 3 receptor subunit alpha IL3RA, is located in the pseudoautosomal region PAR1. In conclusion, we show that WES data can provide insights into eccDNA formation and identify genic eccDNA that may help to identify treatment targets and explain sex differences in the incidence and development of GBM.

Abstract No. 107

Dissecting the role of neuronal cells in glioblastoma progression

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Glioblastoma (GBM) interaction with the surrounding microenvironment and brain resident cells has gained recently increasing relevance. The communication between neurons and glioma cells plays a role in tumor growth and invasion. However, the specific mechanisms involved and how different cell states and mutational background impact on this interaction are still to be defined. To this end, we developed an in vitro model displaying the neuro-tumoral unit where primary human GBM Stem-like Cell Lines (hGSCs) derived from different patient samples were co-cultured with murine primary neurons. After 7 days of coculture, neurons boost glioblastoma cells proliferation and this supportive effect occurred even without physical contact suggesting a putative role of soluble factors. Our results also indicated that the enhanced proliferation was tightly dependent on neuronal activity, with higher and lower proliferative rates associated to enhanced or reduced firing, respectively. To address GBM heterogeneity, a panel of twelve cell lines heterogeneous in transcriptional subtype and in genetic aberrations, was evaluated. Results showed that the vast majority of the cell lines (75%) showed a higher proliferative rate when cultured with neurons ($p < 0.05$) , with the exception of three cell lines that appears to be insensitive. Our functional data were confirmed also by bulk RNA-seq : Ingenuity Pathway analysis revealed the specific enrichment of proliferation and cell division related processes, while neuronal-insensitive lines resulted significantly upregulated in apoptosis and cell death pathways (Z score > 2.0).
These results pinpoint the central role of the neuro-tumoral unit in glioblastoma progression. Indeed, neuronal activity boost cancer cells proliferation through mechanisms that might require paracrine signaling. Further ongoing analysis of neuronal-induced pathways could elucidate the molecular mechanism underpinning neurons-to-glioblastoma communication.

Abstract No. 108

A phenotypic drug discovery platform for combinatorial targeting of cell states and entities

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Glioblastoma (GBM) is a highly lethal brain tumor with limited therapeutic options. The blood-brain barrier (BBB) limits the effectiveness and bioavailability of approved therapeutics and the discovery of new ones. Additionally, GBM consists of heterogeneous, highly plastic cellular entities and states conserved across patients. Tumor cell states change and adapt in response to the current standard of care and infiltration of innate immune cells. For example, myeloid cells drive a mesenchymal state in GBM cells that promotes acquired resistance to therapeutics.

Our lab has developed transcriptional reporters for glioblastoma subtypes named synthetic locus control regions (sLCRs), which inform on cell identity and fate transitions *in vitro* and *in vivo*. Using this tool, we established an *in vitro* phenotypic drug discovery (PDD) platform that recapitulates the interaction between GBM and innate immune cells leading to phenotypic transition and a distinct shift in drug sensitivity in a multicellular 3D model. We show that tumor cells and immortalized microglia co-exist under conditions that enable screening for therapeutic effectiveness of individual drugs over days and their combination with the standard of care. We screened about 1000 small molecules, including lipophilic agents which may possess properties to bypass the BBB. Our platform enables categorization of drug treatments based on tumor cell viability, adaptive cell state changes and the survival of non-transformed innate immune cells. We discovered drugs with the distinct ability to modulate each parameter and novel responses for a clinically relevant drug with potential therapeutic implications.

Hence, our PDD enables data-informed assembly of combinatorial treatments of novel and approved drugs targeting distinct features of the tumor, including a mesenchymal state transition.

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Abstract No. 109

Synthetic genetic tracing of molecular and cellular heterogeneity in Glioblastoma

Matthias Jürgen Schmitt[1,*], *Carlos Company*[1], *Yuliia Dramaretska*[1], *Michela Serresi*[1], *Ben Jiang*[1], *Sonia Kertalli*[1], *Melanie Grossmann*[1], *Maria Pilar Sanchez-Bailon*[1], *Andreas Göhrig*[1], *Iros Barozzi*[2, 3], and *Gaetano Gargiulo*[1]

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Glioblastoma (GBM) remains the most challenging primary solid tumor of the central nervous system despite extensive research on its molecular and cellular properties. Aggressive treatments exist, including surgical resection, radiation, and chemotherapy, but inevitable tumor recurrence and treatment resistance persist due to genetic and cellular heterogeneity, overlaid by phenotypic plasticity.

To improve understanding of GBM heterogeneity, we developed advanced genetic tracing techniques to decipher subtype-specific transcriptional states and intrinsic/non-cell autonomous factors guiding cell fate commitment. Additionally, addressing the demand for sophisticated transcriptional reporters, enabling experimental manipulation and characterization of diseased and developmental cell states, we introduced a computational framework for out method - Logical Design of Synthetic cis-regulatory DNA (LSD). Leveraging phenotypic biomarkers and regulatory networks as input, LSD designs synthetic locus control regions (sLCRs) marking cellular states and pathways. This innovative framework yields flexible transcriptional reporters applicable to diverse biological systems.

We demonstrated mesenchymal GBM adaptation through partially overlapping transcriptional responses involving external signaling and ionizing radiation. Cell fate commitment to a mesenchymal state proved adaptive, reversible, and associated with increased chemotherapy resistance due to crosstalk between innate immune cells and glioma-initiating cells. Our flexible reporters, functional in mouse and human tissues without minimal promoters, have short synthetic DNA-cassettes that can be seamlessly integrated into AAV vectors for gene therapy. In genome-scale CRISPRa screens, sLCRs unveiled both known and novel mesenchymal-GBM cell-state drivers, demonstrating broad applicability for studying complex cell states and transcriptional regulation. Additionally, we expanded our sLCRs to trace epithelial cell responses during SARS-CoV-2 infection, revealing activation driven by interferon-α/β/γ and NF-κB pathways. Drug screens identified JAK-inhibitors and DNA damage inducers as potential modulators of epithelial cell responses to SARS-CoV-2 infection.

In summary, our work advances understanding of GBM heterogeneity and introduces a versatile methodology applicable across diverse research fields, from developmental biology to infectious diseases.

Abstract No. 110

Exploiting metabolic vulnerabilities to sensitize resistant glioblastoma tumor initiating cells toward LSD1-directed therapy

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Despite advancements, effective treatments for glioblastoma (GBM) remain elusive, posing an ongoing clinical challenge. GBM's malignant traits stem from tumor-initiating cells (TICs), driving onset, regrowth, and heterogeneity. Exposure to harsh microenvironmental conditions, such as nutrient deprivation, proteostasis perturbation, hypoxia, and drug treatment, induces molecular and metabolic alterations in GBM-TICs, resulting in the development of stress tolerance and drug resistance traits.

In this context, we have shown that Lysine Specific Demethylase 1 (LSD1) genetic and pharmacological inhibition employing the selective LSD1 inhibitor (LSD1i) impairs TICs ability to restore homeostasis after endoplasmic reticulum (ER) stress or nutrient starvation by hindering a proper Activating transcription factor 4 (ATF4)-dependent integrated stress response (ISR). In line with the GBM inter-tumor heterogeneity, a cohort of patient-derived TICs displayed resistance toward LSD1i.

Our data demonstrate that LSD1i provokes a dysregulation of ER and mitochondria homeostasis triggering physical and metabolic rearrangements of mitochondria in LSD1i-sensitive TICs, ultimately leading to cell death. Contrarily, LSD1i-resistant TICs exhibit an increased capacity to face environmental perturbations due to their metabolic flexibility.

By performing a high-throughput synthetic lethality shRNA screening targeting metabolic genes, we uncovered that Post-GPI Attachment to Proteins 1 (PGAP1), responsible for the proper folding of glycosylphosphatidylinositol-anchored proteins (GPI-APs), serves as a mediator of this intrinsic resistance.

Our findings reveal an innovative role for PGAP1, presenting a novel possibility for precision medicine. By targeting metabolic vulnerabilities, we can sensitize LSD1i-resistant patients, offering a new perspective on treatment strategies. Understanding the intricate interplay between epigenetics and metabolism, particularly in sustaining the plasticity and tumorigenicity of GBM-TICs, holds significant translational promise.

Abstract No. 111

Functional genetic characterization of proneural-to-mesenchymal transition in Glioblastoma

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Glioblastoma (GBM) is the most common and lethal primary brain tumor, with cellular heterogeneity being an enabling feature of its aggressiveness. GBM cell states span across two opposites, proneural and mesenchymal, associated with neural identity and astrocytic inflammatory response, respectively. However, the mechanisms governing proneural-to-mesenchymal transitions (PMT) remain unclear. Here, we employ a cellular model that undergoes PMT in relevant pathophysiological contexts, integrating cell state-specific synthetic reporters, genome-scale functional genetic screens and pathway analysis. Using genome-wide CRISPR-knockout screens, we identify both positive and negative regulators of PMT on a single gene level. Conversely, the convergence of CRISPR-activation screens and patients datasets allowed to identify known and novel bona fide GBM phenotypic drivers. Importantly, pathway analysis of the genome-wide screens led to the discovery of pharmacologic treatments that modulate GBM cell identity and response to its standard of care - concomitant Temozolomide and ionizing radiation treatments in vitro. Overall, our data provide proof of concept for the combination of advanced cell models for PMT, synthetic genetic tracing, and CRISPR/Cas9 screens to connect genetic and pharmacologic perturbations to cell fate decisions underpinning tumor heterogeneity and resistance to treatments.

Abstract No. 112

Uncovering therapeutic combinations for Embryonal tumour with Multilayered Rosettes treatment using a neural progenitor-derived model

Maria Pilar Sanchez-Bailon[1,*], *Heike Naumann*[1], *Melanie Grossmann*[1], *Andreas Göhrig*[1], *Matthias J. Schmitt*[1], *Silke Frahm-Barske*[1], *Annika Zink*[2], *Eilis Perez*[3], *Jorge-Miguel F. Martins*[1], *Anuar Makhmut*[1], *Anne Schöler*[3], *Carlos Company*[1], *Yuliia Dramaretska*[1], *Mina Gouti*[1], *Sebastian Diecke*[1], *Alessandro Prigione*[2], *Fabian Coscia*[1], *Michela Serresi*[1], *Katja von Hoff*[4], *David Capper*[3], and *Gaetano Gargiulo*[1]

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Embryonal tumour with multilayered rosettes, ETMR, is a lethal pediatric brain tumor predominantly affecting children under the age of three, with a poor overall survival. ETMRs are characterized by RNA-binding protein LIN28A expression and genetic aberrations in the microRNA pathway, through amplification of the microRNA cluster on chromosome 19 (C19MC) or DICER1 gene mutations, as well as, increased DNA damage repair pathways. The sensitivity to the combination of topoisomerase and PARP inhibitors, due to the high levels of R-loops, shows an exploitable route targeting DNA damage repair pathways. However, the scarcity of ETMR models due to the rarity of the disease hampers the systematic testing of potentially efficacious treatments. Here, we show that a human neural progenitor-derived cell model, which can be genetically modified to generate model diversity, molecularly resembles BT-183, the most commonly used patient-derived cell line. By using both models, we have set up a drug discovery platform to uncover potent drug combinations anchored to a clinically relevant treatment targeting DNA repair activity. Our findings include a novel triple drug combination demonstrating low-dose efficacy with minimal toxicity in the tested models. This platform enables the discovery of rational drug combinations, enhancing the limited preclinical data available and informing on the design of future studies for ETMR.

Abstract No. 113

Deciphering plasticity in Glioblastoma: towards novel therapeutic strategies

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Introduction

Glioblastoma (GBM) is an aggressive brain cancer occurring in children and adults. Despite intense treatment regimens, patients affected by GBM usually relapse and have an exceedingly poor prognosis. Recent evidence suggests that resistance in GBM stems from intra-tumoral cellular plasticity, whereby GBM cells transition across phenotypic states. Therefore, the aim of this project is to decipher the molecular mechanisms underlying plasticity in GBM, towards the identification of novel vulnerabilities to target.

Results and future perspectives

As a preliminary strategy, we defined intra-tumoral heterogeneity in several 3D systems derived from GBM primary samples. Single-cell RNAseq was employed to evaluate the transcriptional profiles of three GBM samples revealing a high degree of heterogeneity. Moreover, coherently with an existing molecular characterization of cellular states, we selected the cell-surface markers CD44/CD24, and we performed FACS analysis. Specifically, from one sample, clinically defined as Classical, we purified with FACS-sorting four subpopulations to assess their plastic potential. Interestingly, only the CD24 negative subpopulations were able to partially recapitulate the initial heterogeneity of the parental sample over time. Similar results were obtained when we generated single cell derived clones resembling the four cellular states previously identified. Overall, these data indicate an intrinsic phenotypic plasticity. In the next future, to characterize the cellular identity and elucidate the molecular mechanisms underlying plasticity, bulk-RNAseq and proteomic analyses will be conducted on the sorted subpopulations.

Moreover, to understand whether plasticity is a determinant of resistance, we will exploit the results of a high-throughput-drug-screening. Using a library of ~ 2700 FDA-approved compounds, we identified a total of 58 drugs affecting GBM cell's viability. In order to characterize the role of plasticity in the context of GBM drug resistance, we will perform a secondary screening in which selected compounds will be used to assess changes in cellular states and transitions.

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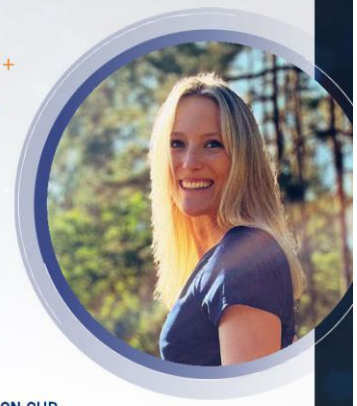

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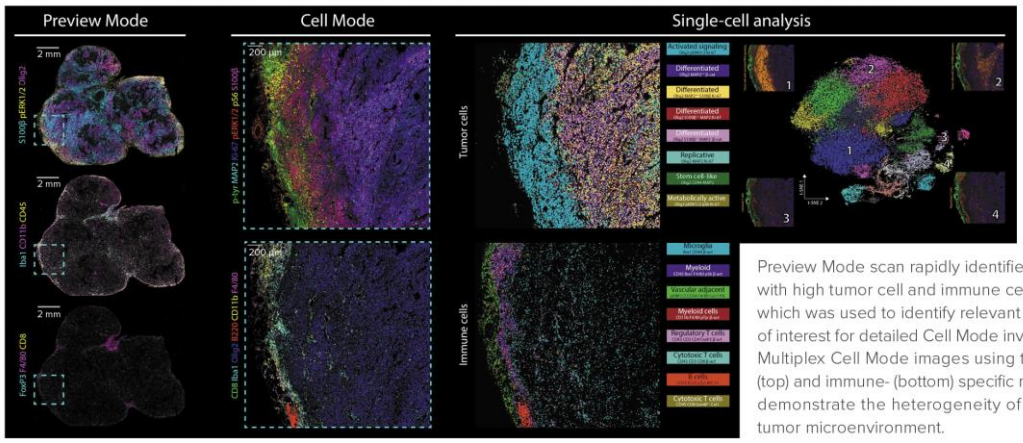

View the study details

Preview Mode scan rapidly identified areas with high tumor cell and immune cell activity, which was used to identify relevant regions of interest for detailed Cell Mode investigation. Multiplex Cell Mode images using tumor- (top) and immune- (bottom) specific markers demonstrate the heterogeneity of the tumor microenvironment.

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