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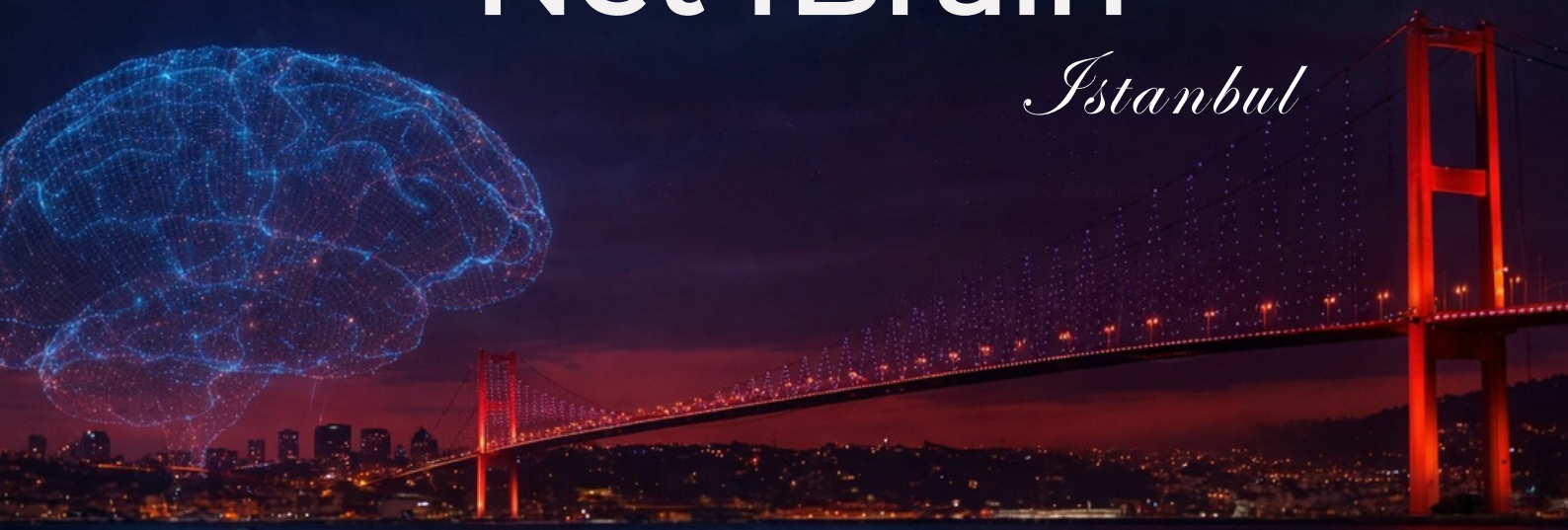

BOOK OF ABSTACTS

June 1–3, 2026

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Abstracts are listed in alphabetical order by presenter surname in each section

SECTION I

STSM Talks

Short-Term Scientific Mission Presentations · June 1, 2026

A diagnostic/prognostic miR-1/-26a-1/-487b signature orchestrates GBM progression by regulating TME and stemness via the SH3PXD2B/EFHD2 axis

AB. Díaz Méndez[1], M. Di Giuliani[1], A. Sacconi[2], G. Ranazzi[3], S. Ramponi[4], V. Barresi[5], A. Silvani[5], A. Di Maio[6], M. Carosi[6], C. Garufi[4], MG. Rizzo[1]

[1]Translational Oncology Research, IRCCS Regina Elena National Cancer Institute, Rome, ITALY
[2]Biostatistics and Bioinformatics, IRCCS Regina Elena National Cancer Institute, Rome, ITALY
[3]Anatomy and Pathological Histology, San Camillo Forlanini Hospital, Rome, ITALY
[4]Medical-Oncology Unit, San Camillo Forlanini Hospital, Rome, ITALY
[5]Neuro-Oncology Unit, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, ITALY
[6]Pathology Unit, IRCCS Regina Elena National Cancer Institute, Rome, ITALY

Glioblastomas (GBMs) are highly invasive brain tumors and challenging cancers for diagnosis and treatment. Current therapies offer only limited benefit, as most GBM patients recur due to the persistence of GBM stem-like cells (GSCs). During progression, GBMs frequently undergo a mesenchymal shift, which is associated with a pro-tumoral microenvironment (TME) that supports GSCs survival, promoting tumor dissemination. Hence, the prognosis of patients remains dismal and effective therapeutic strategies remain urgently needed.

MiRNAs are important players in GBM modulating cancer-related processes, including progression. Recently, we identified a diagnostic/prognostic three-miRNA signature (miR-1/miR-26a-1/miR-487b) differentially expressed in serum of glioma patients according to IDH-mutation. We also uncover that the miRNA signature displays oncosuppressive functions in GBM cells with the most marked inhibitory effects on invasion by targeting SH3PXD2A/SH3PXD2B/EFHD2, which encode for crucial scaffold proteins (TKS5/4 and EFHD2, respectively) involved in invadopodia activity.

Here, we show that the three-miRNA signature is upregulated in paired-primary (pGBM) compared to recurrent (rGBM) patients. In line with the targeting function of miRNAs, two out of three-miRNA signature targets (SH3PXD2B/EFHD2) are downregulated in pGBMs compared to rGBMs and strongly correlated with poor prognosis. Of note, in silico analyses show that pGBMs with higher SH3PXD2B/EFHD2 expression are enriched in immune and angiogenesis related pathways and display higher proportions of immune and endothelial-cells compared to GBMs with lower expression. Moreover, microglia and endothelial cells exposed to supernatant from miR1/26a-1/487b–overexpressing GSCs exhibit decreased levels of SH3PXD2B/EFHD2, along with reduced expression of immunosuppressive and pro-angiogenic markers, respectively, compared to control-GSCs. These data suggest that the miRNA-signature impacts TME by modulating microglia pro-tumoral polarization and angiogenesis. Finally, overexpression of the miR-1/26a-1/487b signature, in paired-primary and -recurrent GSCs, reduces the expression of stem- and epithelial-mesenchymal transition (EMT)-markers, and impairs the tumor-sphere formation capacity. Altogether, our data suggest that miR-1/-26a-1/-487b signature and its targets, SH3PXD2B/EFHD2, impacts on GBM progression by regulating TME, stemness and EMT, thus paving the way for RNA-based complementary therapies in GBM patients.

Integrated in silico strategies for the rational design of isoform-selective SIRT2 inhibitors: 3D-QSAR, molecular docking and machine learning

Aleksandra Ilić[1], Nemanja Djokovic[1], Teodora Djikic[1], Katarina Nikolica[1]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, Belgrade 11000, SERBIA; ailic@pharmacy.bg.ac.rs

Sirtuin 2 (SIRT2) plays a key role in regulating apoptosis, DNA repair, and the cell cycle, making it a promising target for anticancer therapy. However, achieving high isoform selectivity remains challenging due to the conserved nature of the NAD^+-binding site among sirtuin family members. Among known inhibitors, 5-((3-amidobenzyl)oxy)nicotinamide derivatives have demonstrated remarkable potency and selectivity towards SIRT2, providing a solid foundation for further optimization.

In this study, a robust three-dimensional quantitative structure–activity relationship (3D-QSAR) model was developed using a dataset of 86 nicotinamide-based SIRT2 inhibitors and complemented by GRIND-derived pharmacophore modelling. External validation confirmed the predictive reliability of the model, enabling rational design of novel inhibitor candidates.

Furthermore, molecular docking studies on SIRT1–3 isoforms were used to construct machine learning classification models based on Naive Bayes and k-nearest neighbours algorithms for predicting SIRT1/2 and SIRT2/3 selectivity. Docking scores from multiple binding poses were integrated to enhance selectivity prediction accuracy beyond conventional docking approaches.

By combining 3D-QSAR analysis, selectivity modelling, and ADMET predictions, several promising isoform-selective SIRT2 inhibitor candidates were identified. These compounds will be synthesized and evaluated in vitro to validate their biological activity and selectivity, contributing to the development of more effective and selective SIRT2-targeted anticancer therapies.

Adamantane–sclareol hybrid inhibits tumor growth in U87 glioblastoma xenografts

Ema Lupšić[1], Ana Podolski-Renić[1], Pavle Stojkovic[2], Nataša Terzić-Jovanović[3], Fani Koutsougianni[4], Dimitra Alexopoulou[4], Igor M. Opsenica[2], Milica Pešić[1], Konstantinos Dimas[4]

[1]Institute for Biological Research "Siniša Stanković" – National Institute of the Republic of Serbia, University of Belgrade, Despota Stefana 142, 11108 Belgrade, SERBIA
[2]University of Belgrade – Faculty of Chemistry, PO Box 51, Studentski Trg 16, 11158 Belgrade, SERBIA
[3]University of Belgrade – Institute of Chemistry, Technology, and Metallurgy, National Institute of the Republic of Serbia, Njegoševa 12, 11000 Belgrade, SERBIA
[4]Department of Pharmacology, Faculty of Medicine, School of Health Sciences, University of Thessaly, Panepistimiou 3 (Biopolis), 41500 Larissa, GREECE

Glioblastoma is an aggressive brain tumor with limited therapeutic response, largely due to multidrug resistance (MDR). P-glycoprotein (P-gp) reduces intracellular drug accumulation, contributing to MDR. Targeting P-gp and redox homeostasis may improve therapy. In our in vitro study, two adamantane–sclareol hybrids (1 and 2) showed selective cytotoxicity in glioblastoma cells, with enhanced activity in MDR U87-TxR cells, indicating collateral sensitivity. Effects included apoptosis induction, increased reactive oxygen species (ROS), modulation of antioxidant genes, P-gp inhibition, and synergistic interaction with paclitaxel. Therefore, we conducted a preliminary in vivo study with selected hybrid 2 as part of the STSM project funded by Net4Brain.

Male Rag1−/− mice (4–8 weeks) were injected subcutaneously with 1×10^6 U87 cells to establish xenografts. The maximum tolerated dose (MTD) was determined using intraperitoneal doses (7–200 mg/kg; two animals per dose). Tumor-bearing mice were randomized into treatment and control groups (n=5). Hybrid 2 was administered at 7 mg/kg twice, while control group received vehicle. The study was ended when control tumors reached ~11% of body weight.

The MTD was 25 mg/kg. Hybrid 2 at 7 mg/kg strongly suppressed tumor growth, with treated tumors remaining significantly smaller than controls over 12 days. Excised tumors were visibly reduced, and body weight was not adversely affected. In vivo evaluation of 2 demonstrated significant antitumor activity without evident systemic toxicity in the Rag1 xenograft model, supporting its favorable therapeutic profile. On going studies will assess its ability to overcome MDR in resistant glioblastoma models.

EPIKOL, a chromatin-focused CRISPR/Cas9 screening platform, identifies RBBP7 as a selective epigenetic vulnerability in IDH1-mutant gliomas

Sedef Sarac-Yalcinkaya[1,2], Elisa Simone[2], Mina Küçüktaş[1,2], Ebru Yilmaz[1,2], Fidan Seker-Polat[1,2], Alisan Kayabolen[1,2], Ali Cenk Aksu[1,2], Tugba Bagci-Onder[2,3]

[1]Koç University Graduate School of Health Sciences, Istanbul, TÜRKİYE
[2]Koç University Research Center for Translational Medicine, Istanbul, TÜRKİYE
[3]Koç University School of Medicine, Istanbul, TÜRKİYE

Gliomas are the most common incurable malignant tumors of the central nervous system, with an incidence of approximately six per 100,000 individuals. Isocitrate dehydrogenase 1 (IDH1)-mutant gliomas represent a distinct subtype of diffuse gliomas characterized by mutations in the IDH1 gene, leading to accumulation of the oncometabolite D-2-hydroxyglutarate. This metabolic alteration drives widespread DNA and histone hypermethylation, contributing to tumorigenesis and progression, while creating specific epigenetic dependencies.

To interrogate these vulnerabilities, we generated a Cas9-stable A172 cell line pair overexpressing either IDH1-wildtype (WT) or IDH1-R132H mutant (MUT) enzymes via lentiviral transduction. Using our targeted CRISPR/Cas9-based Epigenetic Knock-Out Library (EPIKOL) in these paired cell lines, we identified retinoblastoma-binding protein 7 (RBBP7), a nuclear chromatin remodeling factor, as a regulator of IDH1-MUT glioma cell viability in vitro.

To validate this finding, we assessed the effects of RBBP7 knockout (KO) in the paired A172 cell lines as well as in the primary TS603 glioma cell line. RBBP7 loss significantly impaired proliferation, clonogenic growth, and sphere-forming capacity specifically in IDH1-mutant cells. Ongoing studies aim to define the impact of RBBP7 depletion on cell cycle progression, apoptosis, transcriptomic programs, and in vivo tumor growth. Together, these results nominate RBBP7 as a candidate epigenetic vulnerability in IDH1-mutant glioma and support further mechanistic and therapeutic investigation.

Identifying and creating deep learning models on brain tumour data (MRI) that determine patient survival time and the probability of tumour recurrence

Khuraman Sayin, Ege University, İzmir, TÜRKİYE

The study integrates volumetric MRI data with structured clinical variables to predict overall survival and tumour recurrence risk. Recognizing the challenges of heterogeneous imaging data, incomplete MRI modalities, and class imbalance, the proposed pipeline incorporates strategies for multimodal fusion, missing-modality handling, and balanced learning.

Both survival estimation and recurrence classification were investigated within a unified framework designed to reflect real-world clinical conditions. The results demonstrate that combining imaging and clinical information yields more robust and clinically meaningful predictions compared to imaging-only approaches. Additionally, enabling the use of incomplete imaging data improves dataset utilization and enhances the translational relevance of the model.

Overall, this work establishes a scalable and clinically grounded artificial intelligence pipeline for neuro-oncological prognosis and provides a foundation for future survival-aware and recurrence-risk prediction systems.

SECTION II

Keynotes

June 1–3, 2026

Shaping careers across academia and clinical practice

Claudio Angione[1], Radim Jančálek[2], Milica Pešić[3]

[1]Teesside University, UK
[2]St. Anne's University Hospital, CZECHIA
[3]Institute for Oncology and Radiology, SERBIA

An engaging discussion featuring three professionals who have built their careers across different countries and experiences. Through an open conversation format, the speakers will share their unique career journeys, challenges, and lessons learned while navigating paths in academia and clinical practice. This session offers valuable insights for anyone exploring diverse professional opportunities and international career trajectories, with a particular focus on inspiring and guiding early career researchers in the brain cancer field.

Mapping the rules of glioblastoma using single cell and spatial genomics

Omer Ali Bayraktar

Wellcome Sanger Institute, Hinxton, UK

Cancer cells display heterogeneous and dynamic states in glioblastoma, but how these malignant states arise and whether they follow a tractable cellular trajectory across tumours is poorly understood. Here, we generate a deep single cell and spatial multi-region atlas of glioblastoma that integrates transcriptomic, epigenomic and genomic analysis to comprehensively characterise their tumour heterogeneity. We describe spatially-patterned transitions of malignant cells from dev-like towards glial injury response- and hypoxia-defined states during tumour expansion. This malignant cell trajectory dominates glioblastoma, manifesting across tumours and genetically distinct subclonal lineages that are finely spatially intermixed within tumours. Moreover, this trajectory unfolds across specialised myeloid signalling environments that mirror the spatial compartmentalization of malignant cells. Our findings define a stereotyped trajectory of cancer cells in glioblastoma and unify glioblastoma tumour heterogeneity into a tractable cellular and tissue framework.

Bioengineered perfused human brain microvasculature to model brain tumor and neurodegenerative diseases

Guohao Dai

Department of Bioengineering, Northeastern University, Boston, MA, USA

Blood vessels play an increasingly important role in most human tissue and organ systems. Importantly, vascular niche was found to be a key element of many stem cell environments such as neural stem cells and cancer stem cells. Vascular cells not only form conduits to deliver nutrient and oxygen but also provide instructive signals to control stem cell self-renewal and differentiation, therefore, is critical for tissue regeneration. The mission of Vascular Bioengineering Laboratory is to integrate bioengineering approaches with stem cells and vascular biology to understand blood vessel regeneration and vascular disease processes, and to develop novel therapeutic modalities to treat vascular-related disorders such as cardiovascular, neurovascular and cancer. Toward this goal, our lab has developed the method to bioengineer human brain microvascular network consists of human brain endothelial cells, pericytes and astrocytes. We have shown that interstitial flow promotes lumen formation, interconnectivity and astrocytes association of the bioengineered vasculature and maintains blood brain barrier (BBB) functions. Furthermore, perfused bioengineered vasculature enhances neural stem cell self-renewal and neuronal differentiation and maturation. We have also shown that brain vascular niche supports the infiltrative behavior of glioma stem cells, and glioblastoma dormancy, which contributes to chemo resistance. In this talk, I will present research projects on the bioengineer 3D human brain vascular network and its application in neural stem cell and brain tumor research.

Towards consolidation of mutation-driven cancer progression and drug pharmacokinetics for combination therapies

Bart Westerman[1], Maro Ockhuijsen[1]

[1]Department of Human genetics, RDC ZH2J, Cancer Center Amsterdam CCA, Amsterdam UMC, van der Boechorststraat 6b, 1081 BT Amsterdam, the NETHERLANDS, a.westerman@amsterdamumc.nl

We will present a generative model to predict cancer patient survival based on tumour mutational profiles. By characterizing the structure of all possible mutation combinations—including those that never arise because they are being cleared by the body or are associated with benign tumour behaviour—the model learns latent representations that quantify how individual mutations contribute to tumour progression. This enables identification and prioritization of therapeutic targets by estimating their maximal combinatorial inhibitory effect within the respective mutational landscape. We next construct a comprehensive pharmacokinetic model for small-molecule therapeutics using more than 120,000 data points describing ADMET characteristics. Drug-specific features such as solubility, albumin binding, organ distribution, metabolism, and excretion are integrated and visualized through dimensionality-reduction techniques to reveal dominant determinants of pharmacokinetic behaviour, following an earlier workflow Cornelissen et al. (2022) to generate a broadly applicable pharmacokinetic model for small molecules. Finally, we extend the modelling framework to predict survival outcomes for cancer patients treated with drug combinations. Using an additive model, we estimate the clinical benefit of any of the combination by integrating the individual tumour-volume–reducing effects of each agent. This unified framework will enable to understand mutation-driven disease progression, predicting drug behaviour, and optimizing therapeutic strategies.

SECTION III

Oral Presentations

Invited Talks & Short Talks · June 2–3, 2026

Mechanism-aware deep learning for biomedicine

Claudio Angione

Teesside University, Middlesbrough, UK

In recent biomedical research, deep learning has been widely used for the exploitation of omics data when predicting the cell phenotype, suffering however from a lack of biological interpretability. In parallel, constraint-based mathematical modelling of metabolism has gained popularity due to its scope and flexibility, enabling mechanistic insights into the genotype-phenotype-environment relationship within cells. These two computational frameworks have mostly been used in isolation, having distinct research communities associated with them. However, their complementary characteristics and common mathematical bases make them particularly suitable to be combined. I will describe how machine learning can be combined with constraint-based modelling, discuss the mathematical and practical aspects involved, and show several applications in biotechnology and biomedicine.

Instead of applying machine learning to omics data directly, we propose a multi-view approach merging experimental omics data and model-generated predictions, based on known biochemistry. This architecture can contribute with disjoint information towards biologically-informed and interpretable machine learning, including key mechanistic information in an otherwise biology-agnostic learning process.

The role of biomedical and clinical engineering societies in advancing and promoting cancer research and treatment on a national and international level [Short Talk]

Alkinoos Athanasiou

Aristotle University of Thessaloniki, GREECE

We examine the role of biomedical and clinical engineering societies in advancing and promoting cancer research and treatment, with particular emphasis on large infrastructures and novel investigation modalities at both national and international levels. Using the experience of the Hellenic Society of Biomedical Technology and its collaborations with organizations such as the International Federation for Medical and Biological Engineering (IFMBE), the European Alliance for Medical and Biological Engineering and Science (EAMBES), and the Global Clinical Engineering Alliance (GCEA), we highlight how scientific societies could facilitate interdisciplinary cooperation, policy development, education, and technological innovation in oncology.

We explore the evolution of biomedical engineering initiatives in Europe, including modernization efforts, international partnerships, research infrastructures, and participation in European funding frameworks. Particular attention is given to emerging fields such as artificial intelligence, digital health, health technology assessment, biosensors, and medical imaging, all of which contribute to improved cancer diagnosis and treatment. Lastly, we discuss the strategic importance of developing infrastructure in less developed areas of Europe aiming at creating regional hubs for advanced cancer care and research, acting as catalysts for innovation, capacity building, and international collaboration, and ultimately supporting equitable access to cutting-edge cancer therapies and improved healthcare outcomes.

SVM-RCE-Medoid: A biologically interpretable feature selection approach for glioblastoma transcriptomic data analysis [Short Talk]

Nurten Bulut[1], Bahjat F. Qaqish[2], **Burcu Bakir-Gungor[1],** Malik Yousef[3]

[1]Department of Computer Engineering, Abdullah Gul University, Kayseri, TÜRKİYE
[2]Department of Biostatistics, University of North Carolina at Chapel Hill, NC, USA
[3]Software Engineering Department, Kinneret Academic College on the Sea of Galilee, Zemach, ISRAEL

Gene expression data analysis is challenged by high dimensionality and limited sample sizes, making effective feature selection (FS) strategies essential. Support Vector Machines Recursive Cluster Elimination (SVM-RCE) addresses this issue by iteratively eliminating lowscoring gene clusters formed via K-means clustering. In our previous work, we introduced SVM-RCE with Center Weights (SVM-RCE-CW), where cluster centroids were scored using feature weights obtained from the classifier to improve computational efficiency.

In this study, we propose a medoid-based variant, SVM-RCE-Medoid, in which each cluster is represented by the medoid, the real gene closest to the center, rather than the arithmetic mean (centroid) of cluster members. This modification preserves biological interpretability by selecting actual genes while maintaining the recursive cluster elimination framework. The revised methodology was evaluated on five brain transcriptomic datasets obtained from the Gene Expression Omnibus (GEO). Preliminary results indicate that SVM-RCE-Medoid achieves comparable average classification performance to SVM-RCE-CW in terms of AUC (0.98 vs. 0.998). The proposed approach reduces computational time by approximately 30% on average and selects 44% fewer genes, suggesting improved model compactness and efficiency. These findings highlight the potential of medoid-based cluster representation as a computationally efficient and biologically meaningful refinement for high-dimensional gene expression analysis.

External validation of AI model for glioblastoma survival prediction (GRASP 2)

Thomas Booth

King's College London, UK

Background: Glioblastoma is an aggressive primary brain tumour with poor prognosis despite standard treatment. Early identification of patients unlikely to benefit from standard adjuvant therapy could enable timely transition to alternative or experimental treatments. An artificial intelligence (AI) model developed at King's College London predicts 8-month survival following radiotherapy using post-treatment MRI. Although internally and nationally validated, its international generalisability remains unknown.
Objective: This multi-institutional study aims to externally validate the performance, accuracy, and generalisability of a dockerised AI survival prediction model in independent datasets from international partner institutions.
Methods: Participating centres will retrospectively identify adult patients with IDH-wildtype glioblastoma who underwent surgical resection followed by radiotherapy and post-radiotherapy MRI, including contrast-enhanced T1-weighted and T2-weighted sequences, with confirmed survival status at 8 months. Eligible sites will run the AI model locally using a secure dockerised platform, avoiding the need for central data transfer. Model predictions classifying patients as short-term or long-term survivors will be compared with observed outcomes.
Expected Outcomes: The primary outcome is external validation of predictive accuracy and generalisability across diverse healthcare settings. Secondary outcomes include assessment of variability across institutions and feasibility of decentralised validation workflows.
Impact: Successful validation could support clinical decision-making by identifying patients who may benefit from early treatment modification or clinical trial enrolment. If generalisability is limited, findings will inform subsequent model refinement using local or federated learning approaches.

Current challenges and future directions in brain tumor management [Online Talk]

Angelica Facoetti

CNAO National Center for Oncological Hadrontherapy, Pavia, ITALY

Decoding glioblastoma heterogeneity: from AI-driven multiparametric characterization to clinical translation

Elies Fuster-Garcia[1], Carles López-Mateu[1], Maria Gómez-Mahiques[1], F. Javier Gil-Terrón[1], Víctor Montosa-i-Micó[1], Jaime Agustí Martínez[2], Cristina Alenda González[3], Franciso Martinez-Ricarte[4], Elena Martínez Sáez[4], Vicent Quilis-Quesada[5], Juan M. García Gómez[1]

[1]Institute of Information and Communication Technologies, Universitat Politècnica de València, València, SPAIN
[2]Department of Pathology, Hospital Clínico Universitario de Valencia, València, SPAIN
[3]Biomedical and Health Research Institute of Alicante (ISABIAL), Alicante, SPAIN
[4]Department of Neurosurgery, Hospital Universitari Vall d'Hebron, Barcelona, Spain5Department of Neurosurgery, Hospital Clínico Universitario de Valencia, València, SPAIN

Glioblastoma management is critically hindered by intratumoral heterogeneity and the elusive nature of peritumoral infiltration, which standard imaging fails to fully capture. Our research addresses this challenge by integrating advanced MRI and artificial intelligence to decode tumor phenotypes from vascular, morphological, and biomechanical angles.

We utilize the ONCOhabitats platform (www.oncohabitats.upv.es) to map hemodynamic heterogeneity, distinguishing angiogenic habitats from the critical Infiltrated Peripheral Edema (IPE). Complementing this, our GliomaEdge methodology leverages deep learning to objectively delineate the non-contrast-enhancing tumor (nCET) margin, while novel biomechanical analyses assess peritumoral compression to differentiate infiltrative from proliferative growth patterns.

To bridge the gap between computational innovation and clinical reality, our current initiatives prioritize rigorous validation and technology transfer. Through the ECHIDNA project, we are executing the prospective SINUE clinical investigation with medical device to correlate these imaging biomarkers with multi-omics profiles obtained via image-guided biopsies. Concurrently, the Lab2OR initiative drives the valorization of these technologies by implementing ISO 13485 quality standards and integrating these tools directly into clinicians' workflows.

By transforming complex imaging data into actionable, regulatory-ready Clinical Decision Support Systems (CDSS), we aim to support supramaximal resection planning and personalized therapy, ensuring that AI-driven insights translate into tangible benefits for patient outcomes.

Synthetic genetic tracing of glioblastoma cell states

Gaetano Gargiulo

Max-Delbrück-Center for Molecular Medicine (MDC), Berlin, GERMANY

Cancer therapy has traditionally targeted aberrant genes, proteins or pathways, yet many aggressive tumors persist through phenotypic plasticity and therapy-resistant cell states. Glioblastoma exemplifies this challenge. In my talk, I will discuss an emerging solution: synthetic cis-regulatory DNA that reads transcriptional cell states and converts them into genetic output. Using the recent translational development of synthetic super-enhancer-gated viral immunotherapy for glioblastoma as a case study, I will trace the conceptual path from natural locus control regions and super-enhancers to synthetic genetic tracing for cell-state-selective therapeutic intervention. I will present examples from our own work showing how synthetic genetic tracing offers a broader platform to read, explain and target disease-relevant cell states. Thus, I will argue that synthetic cis-regulatory DNA is poised to move from a biochemical tool to a programmable therapeutic modality for precision oncology.

Probing neural activity and tumor progression in glioma patient-derived zebrafish models [Short Talk]

Nathalie Jurisch-Yaksi[1], Natalia Kostilek[1], Nicole Wadephul-Emden[1], Tejas Borkar[2], Omkar Vinchure[2], Jay Gopalakrishnan[2]

[1]Department of Clinical and Molecular Medicine, NTNU, Trondheim, NORWAY
[2]Institute of Human Genetics, Friedrich-Schiller-Universität Jena, GERMANY

Epilepsy is highly prevalent in glioma, and seizures are often the first clinical manifestation of the disease. Although epilepsy typically does not affect the overall survival of glioma patients, it significantly impairs quality of life and complicates treatment due to drug–drug interactions. Growing evidence suggests that glioma and epilepsy share common pathophysiological mechanisms. For example, glutamate signaling promotes both seizure activity and glioma proliferation, and some antiseizure medications have been reported to slow tumor progression. These observations raise the possibility of developing therapeutic strategies that synergistically target both seizures and tumor growth in glioma patients.

To dissect brain–tumor interactions and to evaluate how brain activity influences tumorigenesis, we have established an in vivo xenograft model in which patient-derived glioblastoma cells are transplanted into the zebrafish brain. This system enables real-time visualization of tumor engraftment, proliferation, invasion, and stromal recruitment, as well as the assessment of tumor-induced changes in behavior and neural activity. In parallel, we are investigating how altered neural activity shapes tumor development. By incorporating patient-derived material into our assays, our goal is to determine whether the zebrafish model can capture patient-specific brain–tumor interactions and thereby support improved prognosis and personalized therapeutic approaches.

Ultimately, our research aims to advance therapeutic strategies that jointly address seizures and tumor progression in glioma, with the broader objective of improving both survival and quality of life for affected patients.

Characterizing and modelling of pediatric high grade tumor microenvironment with single-cell multiomics and spatial transcriptomics

Bozena Kaminska[1], Anna B. Lenkiewicz[1], Szymon Lipiec[2], Patrycja Rosa[1], Maciej Florczyk[1], Monika Dzwigonska[2], Paulina Pilanc-Kudlek[1], Shrinath Dhinapal[1], Wieslawa Grajkowska[3], Katarzyna Kotulska[3], Joanna Trubicka[3], Bozenna Dembowska-Baginska[3], Katarzyna B. Leszczynska[2]

[1]Laboratory of Molecular Neurobiology, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, POLAND
[2]Laboratory of Tumour Hypoxia and Epigenomics, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, POLAND
[3]The Children's Memorial Health Institute, 04-730 Warsaw, POLAND

Pediatric high-grade gliomas (pHGGs) are aggressive brain tumours and the leading cause of deaths in children. They feature unique alterations, such as histone 3 (H3) mutations (K27M, G34R/V) and specific receptor tyrosine kinase fusions, leading to widespread epigenetic dysregulation. Diffuse Midline Glioma (DMG) shows an immunosuppressive tumor microenvironment (TME), but underlying tumor-host interactions are poorly understood. We performed a comprehensive analysis of pHGG TME combining single-cell (sc)RNseq with 40 immune cell markers, Visium spatial transcriptomics, multimodal CODEX (Co-detection by indexing) staining to define diverse immune cell types, cellular states and spatial niches that contribute to a cold TME of pHGGs. DMG and hemispheric tumors have distinct TME regarding relative contribution of myeloid and lymphoid cells with various functionalities. CD45+ cells infiltrating DMGs predominantly comprise of CD11b+/TMEM119+ microglia. Immunosuppressive CD68+Gal3+ macrophages are less frequent, with very few CD3+ T lymphocytes. Microglia expressed transcriptomic programs related to ECM remodeling and angiogenesis, with low expression of chemokines and cytokines, and macrophages displayed phagocytic and immunosuppressive phenotypes. Experiments with co-cultures of wild type or KO DMG K27M cells with microglia uncovered new signaling pathways that could be targeted with small molecules. Immune features of TME have been replicated in experimental NSG mouse models with human tumor cells with or without H3.3 K27M. Overall, our data demonstrate distinct abundance and functionalities of immune cells in epigenetically driven pHGGs which may contribute to the lack of lymphocytes and immunosuppression. The findings indicate a need for immunomodulation to reinvigorate antitumor immunity.

Studies were supported by the EU Cancer Mission Horizon Europe HIT-GLIO project (101136835) founded by the European Health and Digital Executive Agency.

Single-cell molecular profiling in brain cancers: a comprehensive technical review of methodological considerations for patient stratification [Short Talk]

Ebru Kocakaya[1], Yasin Kaymaz[2], Tugba Bagi-Onder[3], Xinzhong Li[4]

[1]Ege University Graduate School of Natural and Applied Sciences, Department of Biotechnology, İzmir TÜRKİYE
[2]Ege University, Faculty of Engineering, Bioengineering, İzmir, TÜRKİYE
[3]Koç University School of Medicine, Istanbul, TÜRKİYE
[4]Centre for Biodiscovery, SHLS Life Sciences, School of Health and Life Sciences, Teesside University, Middlesbrough, UNITED KINGDOM

Single-cell molecular profiling has revolutionized our understanding of brain tumour biology, offering unprecedented resolution of the cellular heterogeneity, microenvironmental landscape, and spatial organization that drive malignancies like glioblastoma. However, while these technologies have generated rich biological insights, most published studies remain exploratory in nature and lack the experimental design required to support robust patient stratification. This review, written within the Net4Brain framework, bridges the gap between biological discovery and clinical utility by defining the criteria for "stratification-grade" datasets. We systematically examine how pre-analytical variables—including surgical sampling strategies, ischemia time, and tissue preservation—introduce bias before data generation, often confounding downstream biological interpretation. Furthermore, we critically evaluate the trade-offs between single-cell (scRNA-seq), single-nucleus (snRNA-seq), and spatial transcriptomics, emphasizing experimental designs that prioritize patient-level replication over total cell throughput to ensure statistical power for grouping. Finally, we address the challenges of multi-center integration and batch correction, synthesizing aggregated methodological insights to transition the field from isolated, descriptive studies toward standardized, reproducible workflows capable of delivering reliable patient stratification.

Building clinically relevant glioma models from patient tissue to trial

Martine Lamfers

Dept of Neurosurgery, Brain Tumor Center Erasmus Medical Center, Rotterdam, NETHERLANDS

Patient-derived glioma tissue provides a powerful foundation for preclinical models that capture inter- and intra-tumoral heterogeneity and enable clinically relevant testing strategies. At Erasmus MC, we have established a comprehensive ex vivo platform of patient-derived glioma model systems spanning increasing levels of biological complexity. These include 2D and 3D cultures of glioma stem cells (GSCs), neurosphere–based co-cultures with autologous peripheral blood mononuclear cells (PBMCs), and organotypic multicellular spheroids (OMS) generated in autologous serum. More recently, we have expanded this platform to include co-cultures of OMS with autologous PBMCs, enabling integrated interrogation of tumor–immune interactions in a patient-specific context.

GSC-based models are currently used for personalized drug screening and are transitioning toward clinical implementation within ongoing and upcoming trials. In parallel, the immune co-culture systems aim to functionally predict patient responses to immunotherapeutic strategies. Molecular and functional signatures derived from these models have been validated using clinical oncolytic virus (OV) trial data, supporting their translational relevance. Prospective validation of the assay will be initiated alongside an upcoming OV trial at Erasmus MC, positioning these patient-derived platforms as predictive tools to guide personalized therapeutic decision-making.

Functional precision medicine screening in patient-derived 3D organoids reveals personalized therapeutic vulnerabilities in high grade gliomas

Anuja Lipsa[1,#], Luca Ermini[1,#], Ann-Christin Hau[1,#], Iryna Krokhmal[1], Bakhtiyor Nosirov[1,2], Reka Toth[2,3], Eliane Klein[1], Anais Oudin[1], Linsey Houben[1], Sabrina Fritah[1,4], Katrin B. M. Frauenknecht[5], Michel Mittelbronn[7], Guy Berchem[6], Frank Hertel[6], Petr V. Nazarov[2,3], Simone P. Niclou[1,7], Anna Golebiewska[1]

[1]NORLUX Neuro-Oncology Laboratory, Department of Cancer Research, Luxembourg Institute of Health, L-1210 Luxembourg, LUXEMBOURG
[2]Multiomics Data Science, Department of Cancer Research, Luxembourg Institute of Health, L-1445 Strassen, LUXEMBOURG
[3]Bioinformatics and AI Unit, Department of Medical Informatics, Luxembourg Institute of Health, L-1445 Strassen, LUXEMBOURG
[4]Cancer RNAs and Epigenetic group, Department of Cancer Research, Luxembourg Institute of Health, L-1210 Luxembourg, LUXEMBOURG
[5]University Medical Center of the Johannes Gutenberg, University Mainz, Mainz, 55131, GERMANY
[6]Centre Hospitalier de Luxembourg, Luxembourg L-1526, LUXEMBOURG
[7]Faculty of Science, Technology and Medicine, University of Luxembourg, L-4367 Belvaux, LUXEMBOURG

Precision medicine has transformed oncology, yet in high-grade glioma molecular advances have not yielded effective targeted treatments. We integrated high-resolution multi-omics with high-throughput functional profiling in patient-derived glioma organoids (PDOs) to refine patient stratification and identify actionable vulnerabilities. We profiled >45 patient-derived high-grade glioma organoid and matched orthotopic xenograft models using targeted DNA-seq, DNA methylation arrays, and bulk RNA-seq. Functional screens were performed in 27 organoid models with a personalized 202-compound library targeting cancer pathways and epigenetic modifiers, and in 20 models with 1,280 FDA-approved drugs. Unsupervised multi-omics factor analysis (MOFA) integrated molecular states with drug responses to identify patient-specific vulnerabilities, followed by time- and dose-dependent validation and biomarker assessment in patient tissues. MOFA identified drug-response subgroups associated with IDH mutation status and pediatric-like DNA methylation signatures. IDH-mutant high-grade astrocytoma models showed preferential sensitivity to histone deacetylase inhibitors, whereas an IDH-wildtype, MYC-amplified glioblastoma model displayed a distinct molecular profile and sensitivity to histone methyltransferase/PRC2-pathway inhibition. Differential efficacy was associated with (epi)genetic and transcriptomic biomarkers and was reproducible across validation assays. In contrast, IDH-wildtype glioblastoma models exhibited heterogeneous responses, and no robust biomarker-defined responder subgroup emerged. Our findings support an integrative functional precision medicine framework to identify clinically relevant patient subgroups in preclinical models, paving the way for tailored interventions in high-grade gliomas, and inform biomarker-guided trial design. Although, larger stratified cohorts, expanded compound libraries, and rational combination strategies will be required to extend this approach to glioblastoma, underscoring the persistent challenge of implementing precision oncology in this disease.

References: Ermini L., Lipsa A., et al. bioRxiv (2025). DOI: 10.1101/2025.09.09.675145
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Polarized light imaging of brain: insights for tumor neurosurgery

Tatiana Novikova[1], Theoni Maragkou[2], Richard McKinley[3], Philippe Schucht[4]

[1]LPICM, CNRS, Ecole polytechnique, IP Paris, FRANCE
[2]Department of Neuropathology, Bern University, SWITZERLAND
[3]Department of Radiology, Bern University Hospital, SWITZERLAND
[4]Department of Neurosurgery, Bern University Hospital, SWITZERLAND

Reliable identification of brain white-matter fiber pathways is a key requirement for accurate brain tumor margin assessment during neurosurgery. We have deployed a custom-built label-free, wide-field polarimetric Mueller matrix imaging system operating in reflection for ex vivo studies of thick sections of human brain tissue and freshly excised animal brain, as well as in vivo measurements of the surgical cavity during open brain tumor surgery. The resulting polarimetric maps provided spatially resolved information on fiber tract architecture related to the optical anisotropy of tumor-free brain white matter. These findings were confirmed by ground-truth histological analysis. Similar contrast patterns were observed in both ex vivo and in vivo proof-of-concept studies, indicating the robustness of the polarimetric imaging modality across different clinical settings. Thus, wide-field imaging Mueller polarimetry holds promise for translation into an intra-operative modality for real-time visualization of healthy white-matter structures, supporting more precise tumor boundary delineation and potentially improving surgical decision-making and patient outcomes. related to the optical anisotropy of tumor-free brain white matter, confirmed by histological analysis. Wide-field imaging Mueller polarimetry holds promise for translation into an intra-operative modality for real-time visualization of healthy white-matter structures.

Glioblastoma phenotypic drift and reversibility: A transport partial differential equation model of tumor resistance [Short Talk]

J.A. Romero-Rosales[1], J. Reveilles[2], C. Ortega-Sabater[3], J. Jiménez-Sánchez[1], G. Seano[2], G.F. Calvo[1]

[1]University of Castilla-La Mancha, Molab, Dept. Matemáticas, Ciudad Real, SPAIN
[2]Institut Curie, Paris, FRANCE
[3]National Cancer Research Centre (CNIO), Madrid, SPAIN

Understanding glioblastoma resistance and recurrence is essential for developing more effective treatments. Mathematical modeling has become a powerful tool for exploring the biological mechanisms underlying these processes and for simulating how different therapies influence tumor evolution.
We study a glioblastoma cell population and analyze how resistance traits evolve under various treatment conditions by focusing on a key marker of a highly invasive and resistant phenotypic state. A central component of the model is the convection term, which represents the epigenetic drift driving cells toward higher resistance in response to therapy. This requires the use of a non-differentiable resistance profile, which complicates the analysis but ultimately enables us to obtain an analytical solution to the system of equations.

Our improved model also captures the reversibility of resistance, describing how cells can transition from senescent states back to more vulnerable phenotypes. With these analytical solutions, we can parameterize the system, fit it to experimental data, and quantify the relative importance of the mechanisms that enable resistance acquisition. Finally, we use the model to explore alternative therapeutic strategies and identify those that may enhance tumor cell vulnerability more effectively.

Control of glioma cell fate by a Golgi ion channel [Short Talk]

M Martins[1,2], L Martin[3], A Mutel[3], MJ Oliveira[4], H Castel[3], AS Fernandes[1], **N Saraiva[1]***

[1]CBIOS, ECTS, Universidade Lusófona, Lisboa, Portugal
[2]Universidad de Alcalá de Henares, Madrid, Spain
[3]Normandie Univ, UNIROUEN, INSERM, IRIB, Rouen, France
[4]i3S, University of Porto, Portugal
*corresponding author: nuno.saraiva@ulusofona.pt

The low survival rate of glioma patients is greatly attributed to the invasion of adjacent tissues and resistance to therapies. The Transmembrane BAX Inhibitor-1 Motif-containing family (TMBIM) includes 6 intracellular ion channels that control processes associated with various cancer hallmarks. Here, we aim to explore the impact of TMBIMs on glioma progression.

Expression of TMBIM1, 4 and 6 is upregulated in gliomas, correlates with tumour grade and is associated with a reduced survival of glioma patients. The high expression of the Golgi-resident protein TMBIM4 near the tumour's vasculature suggests that TMBIM4 is involved in cell invasion along blood vessels. To assess the impact of TMBIM4 expression on glioma cell invasion, 2D/3D cultures of high-grade glioma cells were used as models. The knockdown (KD) of TMBIM4 induces a strong inhibition of cell invasion and tumour growth in an orthotopic mouse model, without affecting cell viability or proliferation. The increased lipid peroxidation and DNA damage observed upon TMBIM4 KD suggest a protective role against oxidative damage. Proteomic analysis of TMBIM4-KO cells revealed a profound reprogramming of amino acid metabolism. Effectively, TMBIM4 KD reduces the expression of enzymes involved in cysteine de novo synthesis, and the addition of cysteine or GSH to TMBIM4-KD glioma cells restored their invasive phenotype.

These results support TMBIM4 as a driver of tumour invasion by maintaining a pool of available GSH, allowing a more efficient maintenance of cellular redox homeostasis. Overall, we propose that TMBIM proteins, particularly TMBIM4, may represent a distinct group of glioma progression biomarkers.

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Programming glioblastoma subtype identity by utilizing synthetic genetic tracing [Short Talk]

Maria Pilar Sanchez-Bailon

Molecular Oncology, Max Delbrück Center for Molecular Medicine (MDC), Berlin, GERMANY

Glioblastoma is a highly aggressive brain tumor characterized by its intra- and inter-patient heterogeneity. The development of accurate glioblastoma models remains a challenge. To screen for factors that enhance the molecular fidelity of the models, we utilized our subtype-specific reporters for the classical, proneural, and mesenchymal subtypes in combination with a compound library in a high-content screening platform. Using this approach, we have established a defined standardized differentiation protocol imparting or restoring glioblastoma identity applicable to engineered, established and patient-derived cell lines. Our higher-fidelity glioblastoma models differentially respond to drugs, highlighting their potential to inform and refine patient-specific treatment strategies.

Bioethical aspects of clinical treatment and management of patients suffering from brain cancer [Short Talk]

Serghei Sprincean

Moldova State University, Institute of Legal, Political and Sociological Research. Academiei str. 3/2, Chisinau, Republic of Moldova, MD-2038

The bioethical aspects of translating fundamental scientific discoveries into better clinical treatment and management of patients suffering from brain cancer refer to a series of normative requirements that need to be implemented to improve the quality of life and dignity of such patients, taking into account as well the well-being of relatives and families of these patients, suffering from brain cancer.

The presentation will contain a series of recommendations for improving the moral, normative and bioethical aspects of research and dissemination of scientific results of studies on the clinical treatment and management of the state of the patients suffering from brain cancer. This will be based on both EU requirements and traditional bioethical principles and elaborations.

Towards European consensus in radiotherapy for brain tumors: a Net4Brain WG5 roadmap

Aleksandar Stepanović[1,2], Alkinoos Athanasiou[3]

[1]Institute for Oncology and Radiology of Serbia, Belgrade, SERBIA
[2]University of Belgrade, Faculty of Medicine, Belgrade, SERBIA
[3]Aristotle University of Thessaloniki, Thessaloniki, GREECE

Radiotherapy is one of the key modalities for the treatment of primary and secondary brain tumors. Considering the latest World Health Organization (WHO) classification of brain tumors from 2021, in addition to the presented and published guidelines for the treatment of certain brain tumors, expert groups in the field of neuro-oncology should periodically review the available guidelines for radiotherapy of brain tumors, including target volume delineation recommendations, dose and fractionation schedule, desirable radiotherapy techniques.

Within the Net4Brain Working Group 5 (WG5), through the collaboration of more than 30 authors from different European centers, an initiative was launched to develop structured, evidence-driven recommendations for radiotherapy in adult diffuse gliomas, meningiomas and brain metastases. The Manuscript would aim to be based on a systematic identification of existing international guidelines and available evidence, while integrating expert consensus and clinical experience. Considering that the decision on the treatment of brain tumors, including radiotherapy, is made based on the expert opinion of various specialties of the tumor board for CNS tumors, the expert consensus of the WG5 group consists of radiation oncologists, neurosurgeons, pathologists, neurologists, molecular biologists, geneticists, radiologists, bioinformaticians, biochemists and others.

The Net4Brain WG5 consensus would represent a unified overview of contemporary recommendations in the field of brain tumor radiotherapy, with a special focus on adult diffuse gliomas, meningiomas and brain metastases, which constitute the most common indications for radiotherapy in neuro-oncology practice and include the majority of clinical scenarios that radiation oncologists routinely encounter.

Plasma denaturation profiles as non-molecular biomarker for brain tumor detection

Philipp Tsvetkov

Institut de NeuroPhysiopathologie, Marseille, FRANCE

Background: Brain tumors, particularly diffuse gliomas and glioblastoma, often require invasive tissue sampling for definitive diagnosis and molecular stratification. Plasma denaturation profiling (PDPs) provides an integrated biophysical readout of the plasma proteome state (including effects of protein composition, interactions, and ligand binding) and can be treated as a digital, non-molecular biomarker.
Methods: We obtained PDPs of recurrent glioblastoma and healthy donors using differential scanning calorimetry (DSC) and differential scanning fluorimetry (nanoDSF) and classified them using machine learning. Across the referenced cohorts, denaturation curves and their derivatives were used as high-dimensional features for automated classification tasks relevant to brain tumor detection and characterization.
Results: DSC revealed disease-associated plasma denaturation signatures in recurrent glioblastoma (n = 17) compared with healthy individuals (n = 10), with cluster structure consistent with clinically relevant heterogeneity; importantly, circulating bevacizumab and carmustine did NOt mask the calorimetric signature, supporting feasibility for monitoring under systemic therapy. In a larger naNODSF study, PDPs from 84 glioma patients and 63 healthy controls were automatically distinguished with up to about 92.5% accuracy using machine learning classifiers. Extending beyond detection, baseline naNODSF profiles in IDH wild-type glioblastoma enabled prediction of EGFR alteration status with 81.5% accuracy using AdaBoost, while MGMT promoter methylation was less separable; early (48 h) post-surgery profiling was not conclusive for immediate monitoring.
Conclusions: PDPs measured by DSC or nanoDSF, combined with machine learning, support a minimally invasive, non-molecular biomarker concept for brain tumor detection and partial molecular stratification. This approach is positioned to complement MRI and clinical assessment for rapid triage and longitudinal follow-up, but requires larger multi-center prospective validation, explicit control of pre-analytical variables, and careful modeling of perioperative inflammatory effects.

Targeting H3K4 trimethylation programs in IDH1R132H-driven gliomagenesis

Marilin S. Koch[1,2], Minh Deo[1,2,5], Claudia Schmidt[4], Adrianna Podolak[1,2], Julian Taranda[1,2,5], Michael S. Hoetker[3], **Şevin Turcan[1,2,5]**

[1]Neurology Clinic and National Center for Tumor Diseases, University Hospital Heidelberg, Heidelberg, GERMANY
[2]Clinical Cooperation Unit Neurooncology, German Consortium for Translational Cancer Research (DKTK), German Cancer Research Center (DKFZ), Heidelberg, GERMANY
[3]Internal Medicine V, Hematology, Oncology and Rheumatology, Heidelberg University Hospital, Heidelberg, GERMANY
[4]Core Facility Unit Light Microscopy, German Cancer Research Center (DKFZ), Heidelberg, GERMANY
[5]Present address: Department of Neurooncology, Center for Neurology, University Hospital Bonn, Bonn, GERMANY

IDH1R132H is the defining mutation of low-grade gliomas (LGGs) and drives broad epigenetic rewiring linked to malignant transformation. Recent studies suggest that the astrocyte-to-LGG fate switch involves redistribution of H3K4 methylation. We asked whether disrupting H3K4 trimethylation via the H3K4 methyltransferase KMT2A is required for IDH1R132H-dependent transformation and whether this pathway yields therapeutic vulnerabilities. In a conditional human astrocyte model expressing IDH1R132H, we inhibited KMT2A with MM-102 and measured marker expression, proliferation, clonogenicity, migration/invasion, and transcriptomic and proteomic changes. Results were validated in patient-derived IDH1R132H glioma lines using shRNA-mediated KMT2A suppression. Epigenetic remodeling was profiled by CUT&Tag and Methylation EPIC arrays, and candidate downstream effectors were tested by siRNA knockdown. KMT2A inhibition reduced expression of an LGG-associated marker and produced broad downregulation of LGG-relevant gene programs. Integrated profiling pointed to altered lipid metabolism and migratory capacity, consistent with impaired invasion, migration, and proliferation. Mechanistically, KMT2A inhibition decreased H3K4me3 at promoters of differentially expressed genes and increased global DNA methylation. We identified SCD as a putative KMT2A-dependent effector; SCD knockdown reduced clonogenicity. In patient-derived models, KMT2A suppression impaired viability and spheroid growth in vitro; however, in an orthotopic TS603 model, KMT2A knockdown shortened survival, underscoring context- and stage-dependent roles. Overall, perturbing KMT2A-mediated H3K4me3 attenuates LGG-like programs in vitro while revealing microenvironment-dependent effects in vivo, motivating cautious, mechanism-guided exploration of KMT2A and downstream metabolic targets. These findings support KMT2A as a key epigenetic node in early IDH1-mutant gliomagenesis, but suggest that therapeutic targeting will require biomarker-based staging and combination strategies in relevant models.

Discovery of unique peptidomimetic compounds via computational modelling against Tau aggregation [Short Talk]

Naz Mina Mert[1], **Kemal Yelekçi**[1]

Kadir Has University, Department of Molecular Biology and Genetics, Istanbul, TÜRKİYE

Alzheimer's disease (AD) is one of the most well-known tauopathies. Tau (tubulin-associated unit) protein is a microtubule-associated protein (MAP) significantly responsible for microtubule assembly, stabilization, and modulation of the normal function of neurons. Most research studies focus solely on the 2N4R isoform of the tau protein, as it is highly expressed in humans and elevated levels are experimentally detected in the brains of adult AD patients. Yet, in a normal human adult brain, 3R and 4R tau protein isoforms are expressed in approximately equal amounts, and the balance between them is of utmost importance. Exploring only the structure and mechanism of the tau protein remains inadequate using experimental methods alone. So, tau-targeting computational drug design-based strategies are highly demanding.

With the utilization of D-I-TASSER, based on machine learning algorithms, the best 3D structures were constructed for each 3R and 4R repeat tau isoform, including 2N3R, 0N3R, 2N4R, and 0N4R. The peptidomimetic compound library was used to screen for novel, high-affinity inhibitors of the regions surrounding repeat sequences implicated in tau protein aggregation in the microtubule-binding region. With the ongoing research the computational predictions will be validated in vitro to test the aggregation-inhibition activities of peptidomimetics for the 2N4R, 0N4R, 2N3R, and 0N3R isoforms.

SECTION IV

Posters

Poster Sessions · June 2–3, 2026

Poster Abstracts

GROUP 1

5th Floor Poster Area · June 2, 2026 · 15:30 – 16:40

1. Uncovering transcriptional dynamics in the cellular hierarchies of glioblastoma to overcome therapeutic resistance

Candela Arribas-Parejas[1,2]; **Diana Aguilar-Morante[1,2]**

[1]Instituto de Biomedicina de Sevilla (IBIS). Hospital Universitario Virgen del Rocio/CSIC/University of Seville, 41013, Seville, SPAIN
[2]Department of Medical Physiology and Biophysics. University of Seville, Seville, SPAIN

Glioblastoma multiforme (GBM) is the most common and lethal primary brain tumour in adults, characterized by remarkable intratumoural heterogeneity, infiltrative growth, and resistance to current therapies. Despite aggressive clinical management—including surgical resection, radiotherapy, and chemotherapy—median survival remains limited to approximately 15 months, and accumulating evidence points to a therapy-resistant subpopulation of Glioblastoma Stem-like Cells (GSCs) that sustains tumour growth, repopulates the lesion after treatment and underpins poor clinical outcome. Data from our ongoing project show that GSCs operate in a hypertranscriptional state, with increased RNA pol II activity, R-loop accumulation and increased Replication–Transcription Conflicts, leading to an increase in the DNA Damage Response (DDR), making them more resistant to the therapy. This hypertranscription can act as a mechanism of oncogene-induced DNA damage, providing a molecular link between upregulation of the transcription machinery and genomic instability in cancer. Still, these mechanisms have not been unexplored in glioblastoma yet. Based on the hypertranscriptional state of GSCs, we study how GSCs can activate specific transcriptional programs to resist the therapy. Using patient-derived primary glioblastoma lines, the study achieves to define active transcriptional landscapes at baseline and after clinically relevant irradiation, pinpointing damage-induced transcriptional “hot spots” enriched in DNA repair and stemness pathways.

2. A modular EGFRvIII-targeted virus-like particle platform for selective glioblastoma elimination

Canan Bayraktar-Odabas[1,2], Ibrahim Sahin Ozyildiz[1,2], Yasemin Holly Özoran[1,2], Tugba Bagci-Onder[1,2,3]

[1]Koç University Graduate School of Health Sciences, Istanbul, Türkiye
[2]Koç University Research Center for Translational Medicine, Istanbul, Türkiye
[3]Koç University School of Medicine, Istanbul, Türkiye

Glioblastoma (GBM) remains refractory to therapy largely due to the lack of tumor-selective cytotoxic strategies. EGFRvIII, a tumor-specific mutant receptor absent from normal tissues, provides a rational entry point for targeted intervention. We engineered a modular virus-like particle (VLP) platform designed to achieve selective glioblastoma elimination through receptor-guided cytotoxic protein delivery.

By systematically testing single-chain variable fragments (scFvs) and peptide ligands against EGFRvIII in paired cell lines, we identified an optimal envelope configuration enabling highly specific recognition and internalization in EGFRvIII-expressing cells. The platform was validated across two endogenously EGFRvIII-positive GBM lines, two engineered overexpression models, and HEK293T controls, allowing assessment under both physiological and ectopic expression contexts.

We packaged distinct cytotoxic cargos, including Cas9 ribonucleoproteins (Cas9-RNPs), caspase-8/3/7 proteins to trigger genome editing or apoptosis. Cas9-RNP VLPs achieved a five-fold increase in editing efficiency in GFP reporter models compared to receptor-negative controls. Caspase-loaded VLPs induced potent, cargo-specific cytotoxicity exclusively in EGFRvIII-positive cells.

This work establishes a customizable, receptor-targeted VLP framework for precision elimination of EGFRvIII-driven malignancies.

3. Chromatin deregulation in glioblastoma and applications for liquid biopsies

Negin Behboodi[1], Amishasingh R. Beeharry[1], Hulkar Mamayusupova[1], Varvara Koraki-Folli[1], Clark Correa[1], Svetlana Gretton[1], Navid Shafiei[1], Thomas Hickson[1], Andra-Ana-Maria Paraschiv[1], Rachael Chapman[1], Isabella Ciuta[1], Madapura M. Pradeepa[1], Victor B. Zhurkin[2], Paul Brennan[3], Vladimir B. Teif[1],*

[1]University of Essex, UK
[2]National Institutes of Health, USA
[3]University of Edinburgh, UK
*Correspondence address: vteif@essex.ac.uk

My project investigates changes in chromatin organisation in glioblastoma and its application to diagnostics using liquid biopsies based on cell-free DNA (cfDNA). I examine differences in nucleosome positioning between glioblastoma patients and healthy individuals using paired tumour and adjacent normal brain tissues and matched blood plasma. This approach aims to improve understanding of genome organisation and identify disease-associated genomic regions. Tissue-derived nucleosome profiles allow us to assess chromatin organisation in living cells, while plasma-derived cfDNA provides a minimally invasive alternative to tissue biopsies, which are often risky in glioblastoma. It enables real-time monitoring of tumour-associated genetic and epigenetic changes, supporting assessment of disease progression and treatment response. Given the high recurrence rate of glioblastoma, cfDNA analysis is particularly valuable for detecting minimal residual disease and early relapse. The project also investigates tumour heterogeneity by comparing cfDNA-derived signals with those obtained from tissue-based biopsies. Additionally, I develop machine learning models using cfDNA-derived features for glioblastoma classification, creating diagnosis frameworks with potential applications beyond this cancer type. Overall, this research integrates genomic and computational methods to advance non-invasive cancer diagnostics and personalised medicine in glioblastoma.

4. Seaweed-derived glioblastoma multiforme inhibitors discovered through an integrated computational approach combining virtual screening, DFT, QTAIM, and toxicity profiling

Avni Berisha[1]

[1]Department of Chemistry, Faculty of Natural and Mathematics Science, University of Prishtina, 10000 Prishtina, KOSOVO e-mail: avni.berisha@uni-pr.edu

Glioblastoma multiforme (GBM) remains one of the most aggressive and difficult-to-treat brain tumors, mainly because of its rapid progression, high molecular complexity, and poor response to current therapies. Therefore, the search for new anticancer compounds with better selectivity and lower toxicity is an important priority in GBM drug discovery. In this work, seaweed-derived metabolites were explored as potential inhibitors of GBM-related molecular targets using an integrated computational approach. The study combined virtual screening, molecular docking, MM-GBSA binding free-energy calculations, density functional theory (DFT), Quantum Theory of Atoms in Molecules (QTAIM), and toxicity profiling. A library of metabolites obtained from the Seaweed Metabolite Database (SWMD) was screened to identify promising marine natural products with potential anticancer activity. The best-ranked compounds showed favorable binding affinities and stable interactions within the target binding sites. Their binding was mainly supported by hydrogen bonds, hydrophobic contacts, π–π interactions, and, in some cases, halogen-bonding contributions. DFT calculations helped explain the electronic behavior of the selected compounds, including their stability, frontier molecular orbital distribution, electrophilicity, chemical hardness/softness, and charge-transfer capacity. QTAIM analysis provided a deeper understanding of the bonding features responsible for ligand–target stabilization by examining electron density and related topological parameters. Toxicity and drug-likeness predictions suggested that several selected compounds may have acceptable safety profiles, although some natural-product structures still require further optimization to improve solubility, molecular size, and pharmacokinetic properties. The study highlights seaweed-derived metabolites as a rich and promising source of bioactive scaffolds for GBM drug discovery. The proposed computational workflow offers a useful starting point for future experimental validation and rational optimization of marine natural products as potential glioblastoma therapeutics.

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5. Towards improving synthetic reporters for phenotypic mapping of glioblastoma atates by LSD+

Ali Osman Cetin[1], Yuliia Dramaretska[1], Gaetano Gargiulo[1]

[1]Max-Delbrück-Center for Molecular Medicine, Robert-Rössle-Str. 10 13125 Berlin, GERMANY

Cell types and cell states are fundamental units in issues. 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. In conclusion, LSD+ not only advances synthetic reporter design but also enhances its scalability, improving the functional characterization of complex cell identities and states.

6. Combining focused ultrasound with immunotherapy against melanoma brain metastasis

Vinton Cheng, Catherine Gorick, Andrew Thim, Victoria Breza, G. Wilson Miller, Tim Bullock, Richard Price

University of Leeds, UK
University of Birmingham, UK
University of Virginia, USA

Background: Brain metastases (BrM) are common in patients with cutaneous melanoma, causing significant morbidity and mortality. We applied focused ultrasound (FUS) with microbubbles (MB) for blood-brain barrier (BBB) opening, combined with anti-CD40 immunotherapy, in a two-site model of metastatic melanoma to assess the immune response against BrM.
Methods: B16F1cOVA cells were subcutaneously (s.c.) inoculated in the right flank of C57BL/6 mice (day 0), followed by intracranial (i.c.) inoculation (day 5). We tested: (1) systemic anti-CD40 alone; (2) systemic anti-CD40 with FUS+MB and (3) IgG with FUS+MB. Systemic anti-CD40/IgG was administered on days 10 and 12; MRI-guided FUS with MB was co-administered on day 12. Immune cell infiltration was analysed by spectral flow cytometry.
Results: Anti-CD40 with/without FUS+MB did not improve survival compared to IgG. Systemic aCD40 increased circulating myeloid and dendritic cells (DC) with reductions in T and NK cells compared to IgG. FUS+MB for BBB opening, increased SIRPa+ DC and reduced XCR1+ DC in i.c. Tumours of anti-CD40-treated animals vs. IgG group; also observed in s.c. Tumours. Moreover, i.c. Tumours in animals that received aCD40 with FUS+MB had more bone marrow derived macrophages compared to the aCD40 alone and IgG cohorts.
Conclusion: These insights will allow us to optimise the intercalation of FUS+MB BBB opening with novel immune modulating agents against BrM.

7. BRD9 inhibition enhances radiotherapy response in glioblastoma by disrupting MYC-driven translational programs

Nareg Degirmenci[1], Serdar Aksel Çelikkol[1], Beyza Nur Köseoğlu[1], Sedef Saraç Yalçınkaya[1], Martin Philpott[2], Adam Cribbs[2], Uğur Selek[3,4], Tugba Bagci-Onder[1,4]

[1]Brain Cancer Research and Therapy Laboratory, Koç University Research Center for Translational Medicine (KUTTAM), Koç University, Istanbul, TÜRKİYE
[2]Botnar Research Centre, Nuffield Department of Orthopedics, Rheumatology and Musculoskeletal Sciences (NDORMS), University of Oxford, Oxford, UK
[3]Department of Radiation Oncology, Koç University School of Medicine, Istanbul, TÜRKİYE
[4]Koç University School of Medicine, Istanbul, TÜRKİYE

Radiotherapy (RT) remains a cornerstone of glioblastoma treatment; however, intrinsic and acquired radioresistance severely limit therapeutic efficacy and contribute to tumor recurrence. Adaptive transcriptional and translational programs activated after ionizing radiation (IR) enable tumor cells to survive genotoxic stress. Identifying molecular regulators that can prime GBM cells for enhanced radiation response represents a critical unmet need.

An epigenetic small-molecule library screen (125 compounds) combined with IR (4 Gy) identified bromodomain-containing protein 9 (BRD9) inhibition as a prominent radiosensitizing strategy. Pharmacological inhibition using structurally distinct BRD9 inhibitors and CRISPR/Cas9-mediated BRD9 loss significantly enhanced IR-induced clonogenic death in glioblastoma cells, while non-malignant astrocytes were minimally affected, indicating tumor-selective vulnerability. BRD9 inhibition primed glioblastoma cells for radiotherapy by increasing apoptotic fractions and delaying resolution of γH2AX and 53BP1 foci following irradiation.

Transcriptomic profiling revealed marked downregulation of MYC target genes, ribosomal biogenesis pathways, and translational programs upon BRD9 perturbation. Consistently, global protein synthesis was reduced upon BRD9 inhibition, suggesting that BRD9 activity sustains MYC-dependent translational capacity required for radiation tolerance. Long-term IR-surviving tumor cell populations exhibited elevated BRD9 expression, supporting its role in adaptive radioresistance. Importantly, ectopic MYC overexpression restored clonogenic survival despite BRD9 inhibition, reversing the primed radiosensitive state.

These findings demonstrate that BRD9 inhibition primes glioblastoma cells for radiotherapy by suppressing MYC-driven translation and highlight a promising radiosensitization strategy.

8. Identification of a kinase-autophagy signaling hub as a therapeutic vulnerability in pediatric glioma

Suheda Duran[1], Gamze Deveci[1], Nehir Dalgic[1], İbrahim Bayramoğlu[2], Jörn Dengjel[2], Devrim Gozuacik[1,3]

[1]Koç University Research Center for Translational Medicine (KUTTAM), Istanbul, TÜRKİYE
[2]University of Fribourg, Department of Biology, Fribourg, SWITZERLAND
[3]Koç University School of Medicine, Department of Medical Biology, Istanbul, TÜRKİYE
Correspondance: dgozuacik@ku.edu.tr

Pediatric glioma remains a devastating malignancy characterized by a profound lack of effective therapeutic interventions. This study identifies a specific stress-responsive kinase as a pivotal regulator of autophagy in pediatric glioma cells. Autophagy is a cellular recycling process essential for maintaining homeostasis and ensuring tumor survival under nutrient, energy, and amino acid deprivation. Through SILAC-MS/MS, co immunoprecipitation, and colocalization analyses, we demonstrated that this kinase directly interacts with the key autophagy proteins, an association that is significantly enriched during autophagy-inducing stress. Functional validation reveals that the kinase is essential for pathway activation, as its knockout or knockdown significantly impairs autophagy, whereas its overexpression is sufficient to initiate the process under basal conditions and amplify it under stress. Furthermore, our proteomics-based mapping of the kinase interactome and its phosphorylation targets uncovered several novel partners. By establishing this kinase as a central modulator of autophagic flux, our findings provide critical insights into basic cellular mechanisms and highlight the targeting of this kinase-autophagy axis as a promising therapeutic strategy for the treatment of pediatric glioma.

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9. Early detection of glioblastoma progression using time-varying mixed models

Dário Ferreira[1,*], Dhruba Das[2], Jondeep Das[3], Asmita Tamuli[2], Sandra Ferreira[1]

[1]Department of Mathematics and Center of Mathematics and Applications, University of Beira Interior, PORTUGAL
[2]Department of Statistics, Dibrugarh University, INDIA
[3]Department of Statistics, Bhattadev University, INDIA
*Corresponding author: dario@ubi.pt

Clinical management of high-grade gliomas is often hindered by the detection lag inherent in response assessment criteria that rely on static volumetric thresholds (e.g., RANO). We propose a dynamic monitoring framework that combines a time-varying linear mixed model (TVLMM) with residual-based change-point analysis to flag early signs of treatment failure and progression-related regime shifts. Patient-specific growth dynamics are modeled through a stochastic drift with time-varying uncertainty (ARIMA–GARCH-style), aiming to disentangle measurement variability from sustained biological acceleration by learning both an expected trajectory and its predictive band.

We assess the approach on (i) simulated longitudinal trajectories and (ii) real-world glioblastoma MRI-derived volumetric series (necrotic tumor core). In simulation, TVLMM-based monitoring flags the onset of accelerated growth at Month 19, compared with Month 23 for a static 25% threshold rule, yielding a 4-month lead-time advantage. Real-world trajectories qualitatively demonstrate how the method highlights abrupt recurrences and aggressive regrowth even under limited longitudinal sampling.

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10. Clinical and molecular determinants of survival in glioblastoma: A reproducible analysis using an open clinical dataset

Sandra S. Ferreira[1], Dário Ferreira[1]

[1]Mathematics Department and Center of Mathematics and Applications, University of Beira Interior, Covilhã, PORTUGAL

Glioblastoma research is dominated by complex, non-transparent models and institution-specific cohorts, limiting clinical uptake and cross-center comparability. This work presents an innovative, reproducible survival and progression analysis framework tailored for neuro-oncology practice. Using open clinical-molecular glioblastoma data, the approach integrates standard time-to-event methods with automated, interpretable reporting focused on treatment pathways and equity assessment. The framework is designed for direct adoption by clinical teams and seamless replication across cancer centers and countries, supporting harmonized evidence generation in brain cancer research.

11. CDC7 inhibition with PHA-767491 modulates Temozolomide resistance pathways in T98G glioblastoma cells

Kimia Seifollahi[1], **Öykü Gönül Geyik[2]**

[1]Medical Biology and Genetics Doctoral Program, Institute of Graduate Education, Istinye University, Istanbul, TÜRKİYE
[2]Department of Medical Biology, Faculty of Medicine, Istinye University, Istanbul, TÜRKİYE

Temozolomide (TMZ) remains a backbone therapy for glioblastoma (GBM), yet intrinsic or acquired resistance markedly limits benefit. Resistance is shaped by DNA damage tolerance/repair capacity and drug efflux mechanisms. CDC7 is a replication-initiation kinase that links DNA replication to checkpoint signaling and may represent a druggable vulnerability in therapy-resistant GBM.

Aim of this study is to investigate whether the small-molecule CDC7 inhibitor PHA-767491 alters TMZ resistance–associated mechanisms in the TMZ-resistant T98G GBM cell line. T98G cells are treated with TMZ, PHA-767491, and the TMZ+PHA-767491 condition across defined concentration ranges and timepoints. Treatment response is quantified by the sulforhodamine B (SRB) assay to determine dose–response behavior and IC_{50} values. To profile molecular changes linked to TMZ refractoriness and GBM pathobiology, total RNA is isolated from selected conditions followed by cDNA synthesis and qRT-PCR analysis of resistance and regulatory nodes, including MGMT, drug transporters (ABCB1, ABCG2), and cell-cycle/DNA damage–related regulators (TP53, E2F7).

This work integrates a TMZ-resistant GBM preclinical model with targeted tumour profiling to define whether CDC7 inhibition reshapes key resistance pathways and improves TMZ responsiveness. The results are expected to provide mechanistic rationale for CDC7-focused strategies and guide subsequent validation in more complex GBM systems.

Keywords: glioblastoma, temozolomide, resistance, CDC7, PHA-767491, MGMT, ABCB1, ABCG2, qRT-PCR.

12. Learning glioblastoma immune phenotypes from whole-brain MRI using a hybrid deep learning radiogenomic framework: DECIPHER-GBM study

Prajwal Ghimire

King's College London, London, UK

Background: Glioblastoma is characterized by profound heterogeneity in its immune microenvironment, with tumor-associated macrophages representing a dominant and clinically relevant immune population. This immunological landscape is increasingly recognized as a critical determinant of response to emerging immunotherapies. However, direct immune profiling remains invasive and poorly suited to longitudinal or population-level assessment. Imaging-based immune biomarkers remain constrained by region-of-interest radiomics and limited external validation. We developed DECIPHER-GBM, a radiogenomic AI framework leveraging deep learning to learn tumor immune microenvironment phenotypes non-invasively from standard-of-care multiparametric whole brain 3D MRI of IDH-wildtype glioblastoma patients.
Material and Methods: We performed a retrospective multicenter study using curated open-access anonymized imaging and genomic data from five independent datasets. Imaging data consisted of pre-processed whole brain 3D MRI sequences. Seventeen deconvoluted transcriptomics-derived immune labels were generated using pan-cancer and glioblastoma-specific signatures as reference standards respectively and binarized via Gaussian mixture modelling. This was implemented using 3D hybrid autoencoder-classifier architecture for immune label predictions. Six models were trained, validated and tested with three cross-cohort holdout strategies using three independent holdout datasets.
Results: Across cohorts, tumor associated macrophages emerged as the most robust and transferable immune phenotype. Models trained on labels from glioblastoma-specific signatures demonstrated consistently lower false-negative rates for TAMs-high tumors under domain shift compared with pan-cancer signature derived models. Probability density and calibration analyses revealed structured confidence distributions for TAMs predictions. Bootstrap analysis confirmed statistically significant improvements in balanced accuracy using models derived from glioblastoma-specific signature labels.
Conclusion: Whole-brain autoencoder-based modelling enables non-invasive, domain robust inference of global macrophage burden in glioblastoma. These findings establish TAMs as a generalizable imaging biomarker and support DECIPHER-GBM as a scalable framework for immune phenotyping, with implications for patient stratification in immunotherapy trials.

13. Canonical histone H3 variants are linked to cell cycling in glioblastoma

Luna Guerra-Núñez[1], Anabel García-Heredia[1], Juan M. Molina[2], Paula Martín-Climent[1], Artemio Payá[2], María Fuentes-Baile[4,5], Miguel Saceda[4,5], Juan F. Gallego-Serna[1,4], Cristina Alenda[2], Luis M. Valor[1,4]

[1]Research Laboratory, Dr. Balmis General University Hospital, Alicante Institute for Health and Biomedical Research (ISABIAL), 03010 Alicante, SPAIN
[2]Department of Anatomical Pathology, Dr. Balmis General University Hospital, Alicante Institute for Health and Biomedical Research (ISABIAL), 03010 Alicante, SPAIN
[3]Department of Neurosurgery, Dr. Balmis General University Hospital, Alicante Institute for Health and Biomedical Research (ISABIAL), 03010 Alicante, SPAIN
[4]Institute of Research, Development, and Innovation in Healthcare Biotechnology in Elche (IDiBE), Miguel Hernández University, 03202 Elche, SPAIN
[5]Research Unit, Fundación para el Fomento de la Investigación Sanitaria y Biomédica de la Comunidad Valenciana (FISABIO), 03203 Elche, SPAIN
[6]Molecular Genetics Unit, Elche University General Hospital, 03203 Elche, SPAIN

Glioblastomas (GBM) are the most prevalent primary tumours in the central nervous system, which are characterized by a special aggressivity and poor outcome that is difficult to tackle due to their high heterogeneity across patients. Epigenetic dysregulation is of paramount importance to maintain the proliferative and self-perpetuation properties of tumoural cells and can be reversed by pharmacological means. In this work, we have examined the expression and function of the main histone H3 variants (the canonical H3.1/H3.2 and the alternative H3.3) across diverse types of gliomas and glioneural tumours with particular emphasis in GBM. To this aim, we used our in-house multiomics datasets from FFPE-derived tumours and we manipulated GBM cultures derived from patients. As a result, we determined that a high expression of canonical H3 variants was generally associated with brain tumours of high aggressiveness and lower survival; and correlated with the induction of genes related to mitosis and nucleosome assembly. Blocking the cell cycle in cultured cells dramatically lowered the expression of H3-1/H3.2 genes and, conversely, interfering H3.1 transcripts reduced cell viability. Contrary to expectations, the replication-independent H3.3 has not simply counteracting activities. The study presented here opens the possibility for canonical histone variants as biomarkers of therapeutically targetable cells.

14. Epigenetic alterations in U87 glioblastoma cells following treatment with novel curcumin analogs

Yasemin Oyacı[1], **Başak Varol Günçer[2]**, Bilge Özerman Edis[2], Funda Özkök[3], Sacide Pehlivan[4]

[1]Department of Basic Sciences, Istanbul Nişantaşı University, Istanbul, TÜRKİYE
[2]Department of Biophysics, Istanbul Faculty of Medicine, Istanbul University, Istanbul, TÜRKİYE
[3]Department of Chemistry, Faculty of Engineering, Istanbul University-Cerrahpaşa, Istanbul, TÜRKİYE
[4]Istanbul Faculty of Medicine, Istanbul University, Department of Medical Biology, Istanbul, TÜRKİYE

Glioblastoma (GBM) is the most aggressive primary malignancy of the central nervous system. Despite standard treatment including maximal surgical resection followed by radiotherapy and chemotherapy with Temozolomide (TMZ), median survival remains limited to 12–18 months. Genome-wide hypomethylation is a hallmark of many cancers, including GBM, contributing to genomic instability and tumor progression. Targeting epigenetic dysregulation may therefore represent a novel therapeutic strategy.

This study aimed to investigate the effects of newly synthesized curcumin derivatives on global DNA methylation in U87 glioblastoma cells. U87 glioblastoma cells were treated with curcumin, P1 (a curcumin ester analogue), or P1-M (a copper complex of the curcumin ester analogue) at concentrations of 10–20 µM, either alone or in combination with TMZ (100 µM). Global DNA methylation levels were determined by measuring 5-methylcytosine (5-mC) using a colorimetric assay. Untreated cells served as controls.

Control U87 cells exhibited low baseline methylation levels (5-mC = 0.19%), consistent with GBM-associated hypomethylation. Treatment with curcumin and its derivatives significantly increased global methylation levels, with the most pronounced effect observed in the P1-M (10 µM) group (5-mC = 0.36%). Although TMZ alone or in combination with curcumin or P1 reduced global methylation levels, the combination of TMZ with P1-M (100+10 µM and 100+20 µM) uniquely counteracted this reduction and increased 5-mC levels. The novel curcumin analog P1-M effectively reverses global DNA hypomethylation in U87 glioblastoma cells and maintains this effect in combination with TMZ. Epigenetic alterations via curcumin-based analogs may represent a promising adjunct therapeutic strategy in GBM management.

15. Blood–Brain Barrier-targeted nanoparticles for peptide delivery to glioblastoma: An in vitro and in vivo study

Teresa Abengoza, Marcos Bazarra-Barreiros, Ramón Iglesias-Rey, **Pablo Hervella**

Neuroimaging and Biotechnology Group, Health Research Institute of Santiago de Compostela (IDIS), Santiago de Compostela, SPAIN

Introduction: Glioblastoma (GBM) is the most aggressive primary malignant brain tumor in adults, with a median survival of less than two years. A major limitation in GBM therapy is the inability to target infiltrative tumor cells beyond the tumor core due to the blood–brain barrier (BBB). Here, we developed lipid nanoparticles (LNPs) mimicking low-density lipoproteins for the delivery of model peptides targeting the glioma extracellular matrix. The ability of these nanoparticles to cross the BBB, accumulate in tumors, and release their payload was evaluated using in vitro assays and in vivo imaging.
Methods: LNPs were formulated by ethanol injection, and peptides were encapsulated via reversible esterification of the N-terminal amino acid. Nanoparticles were characterized by dynamic light scattering, zeta potential, and transmission electron microscopy. Cellular uptake was assessed in glioblastoma, endothelial, astrocyte, and neuroblastoma cell lines. Biodistribution and tumor accumulation were studied in the GL261 murine glioblastoma model. BBB transport was analyzed by intravital multiphoton microscopy.
Results: LNPs (~30 nm) efficiently incorporated extracellular-matrix-targeting peptides. BBB-targeted LNPs showed selective uptake in neuroblastoma and astrocytes and significantly increased accumulation in brain tumors compared with non-targeted nanoparticles. Vascular accumulation was observed 1 h post-injection and persisted up to 48 h.
Conclusions/Impact: BBB-targeted LNPs are effective carriers for peptide delivery to brain tumors, with LDL-receptor-targeting peptides enhancing BBB penetration and tumor accumulation.

16. Benchmarking vision-enabled multimodal large language models for clinical reasoning in brain tumor neuroimaging

Ilinka Ivanoska, Katarina Trojachanec Dineva, Stefan Andonov, Ivan Kitanovski, Sasho Gramatikov, Monika Simjanoska Misheva, Kostadin Mishev

Faculty of Computer Science and Engineering, Ss. Cyril and Methodius University in Skopje, NORTH MACEDONIA

Brain tumors represent a major clinical challenge due to their heterogeneity, complex imaging characteristics, and critical impact on patient outcomes. Accurate interpretation of neuroimaging is essential for diagnosis, subtype differentiation, and treatment planning. Recent advances in multimodal large language models (LLMs) have opened new possibilities for image-based decision support. However, their reliability, calibration, and practical trade-offs in brain tumors neuroimaging remain poorly understood and underexplored. In this work, we present a systematic comprehensive benchmarking

Study of 20 vision-enabled multimodal large language models for 2D neuroimaging analysis of brain tumors from multiple providers, using a curated collection of publicly available datasets. Rather than describing the problem as isolated tasks, we evaluated a set of clinically significant output fields, including primary diagnostic classification, identification of diagnostic subtypes, and recognition of imaging attributes. Model performance is evaluated using predictive performance metrics with abstention handling. Zero-shot and few-shot prompting are systematically compared to quantify performance gains and associated computational and cost trade-offs. Overall, this benchmark provides clinically grounded insights into the performance, reliability, and efficiency of multimodal large language models for brain tumor neuroimaging, contributing toward standardized evaluation and safer deployment of multimodal AI in neuro-oncological decision-support settings.

17. Biomarkers for glioblastoma resistance to radiotherapy: Metabolomic study of cells, culture media and small extracellular vesicles

Salomé Araujo-Abad[1], Marina Botello-Marabotto[4,5,6], María Fuentes- Baile[3], Miguel Saceda[2,3], Ramón Martínez-Máñez[4,5,6,7], M. Carmen Martínez-Bisbal[4,5,6,8]*, **Camino de Juan Romero[2,3]***

[1]Cancer Research Group, Faculty of Engineering and Applied Sciences, Universidad de Las Américas, 170124 Quito, ECUADOR
[2]Unidad de Investigación, Fundación para el Fomento de la Investigación Sanitaria y Biomédica de la Comunidad Valenciana (FISABIO), Hospital General Universitario de Elche, Camí de l'Almazara 11, Elche, 03203 Alicante, SPAIN
[3]Instituto de Investigación, Desarrollo e Innovación en Biotecnología Sanitaria de Elche (IDiBE), Universidad Miguel Hernández, Avda, Universidad s/n, Ed. Torregaitán, Elche, 03202 Alicante, SPAIN
[4]Instituto Interuniversitario de Investigación de Reconocimiento Molecular y Desarrollo Tecnológico (IDM), Universitat Politècnica de València - Universitat de València, Camino de Vera, s/n, 46022 Valencia, SPAIN
[5]CIBER de Bioingeniería, Biomateriales y Nanomedicina, Instituto de Salud Carlos III, Madrid, SPAIN
[6]Unidad Mixta de Investigación en Nanomedicina y Sensores, Universitat Politècnica de València – Instituto de Investigación Sanitaria La Fe, 46026 Valencia, SPAIN
[7]Unidad Mixta UPV-CIPF de Investigación en Mecanismos de Enfermedades y Nanomedicina, Universitat Politècnica de València, Centre de Investigació Príncipe Felipe, 46012 Valencia, SPAIN
[8]Departamento de Química Física, Universitat de València, C/ Doctor Moliner, 50, 46100 Burjassot, Valencia, SPAIN
*Authors to whom correspondence should be addressed.

Glioblastoma (GBM) is the most aggressive cancer of brain origin, the late diagnosis and drug resistance acquisition are some of the main problems. In this work, we have used NMR spectroscopy to perform a complex metabolomic study to search for GBM biomarkers that could also serve for the identification of resistance to therapy. We have obtained cells, small extracellular vesicles (EVs) and culture media from seven patient-derived GBM cell lines, and we have analyzed the metabolomic differences in these samples according to the resistance to radiotherapy. This study integrates previous data from genomic studies on GBM cell lines with new results obtained from metabolomics studies. Using metabolomics, we provided data that GBM-derived small EVs contain metabolites, amino acids, lipids, and TCA-cycle intermediates that are used by GBM cancer cells and promote tumor growth under nutrient-deprivation conditions. In the future, the combined information obtained from these types of studies will be used to identify potential biomarkers for liquid biopsies in these tumors. It will be possible to apply these markers to the stratification of patients, guide their treatment, and identify new therapeutic targets.

Keywords: Metabolomics, NMR spectroscopy, glioblastoma, small EVs, biomarkers.

18. Identification and therapeutic validation of a glioblastoma molecular signature using bioinformatics

Víctor M. Barberá[2,3,+], Nuria Termes-Sabater[1]+, Julia Ruiz-Agulló[1,2], Luis M. Valor[1,4], Javier Morales[5], Camino de Juan Romero[1,2,*]

[1]Instituto de Investigación, Desarrollo e Innovación en Biotecnología Sanitaria de Elche (IDiBE), Universidad Miguel Hernández, Avda. Universidad s/n, Ed. Torregaitán, Elche, 03202 Alicante, SPAIN
[2]Unidad de Investigación, Fundación para el Fomento de la Investigación Sanitaria y Biomédica de la Comunidad Valenciana (FISABIO), Hospital General Universitario de Elche, Camí de l'Almazara 11, Elche, 03203 Alicante, SPAIN
[3]Unidad de Genética Molecular, Hospital General Universitario de Elche, Camí de l'Almazara 11, Elche, 03203 Fundación para el Fomento de la Investigación Sanitaria y Biomédica de la Comunidad Valenciana (FISABIO), Elche, Alicante, SPAIN
[4]Unidad de Investigación, Instituto de Investigación Sanitaria y Biomédica de Alicante (ISABIAL), Hospital General Universitario Dr. Balmis, Av. Pintor Baeza 12, 03010 Alicante, SPAIN
[5]Applied Statistical Unit, Centro de Investigación Operativa, Universidad Miguel Hernández, Avenida de la Universidad s/n, 03202-Elche, Alicante, SPAIN
+These authors contributed equally to the work.
*Correspondence: m.juan@umh.es

Glioblastoma (GBM) is a highly aggressive and lethal brain cancer with limited treatment options. Due to the heterogeneity of the tumor, standard treatments including surgery, radiotherapy and chemotherapy are insufficient. Therefore, finding a new approach to treating this disease is a challenge in medical research.

This work identified a molecular signature associated with cancer aggressiveness that could serve as biomarkers for earlier diagnosis and prognosis. By comparing clinicopathological data and genetic profiles of 24 GBM patients with low-grade glioma patients, 13 genes related to aspects of tumor development were selected among the differentially expressed genes in GBM. These genes are also potential targets for new treatments. We have identified FDA-approved compounds that target some of these genes and are currently being used to treat other diseases, facilitating their potential application in the treatment of GBM in an exercise of drug repurposing. To validate these findings, we tested epirubicin that markedly reduced cell viability, particularly in HGUE-GB39 cells. Methotrexate showed limited efficacy in GBM cells but was effective in colon adenocarcinoma cells, a difference associated with reduced SLC19A1 expression and increased DHFR levels in GBM.

Overall, this study not only identifies prognostic biomarkers and therapeutic targets but also supports the feasibility of drug repurposing in GBM while underscoring the importance of biomarker-guided strategies to advance toward more personalized and effective therapies.

19. Speedy/RINGO shapes DNA damage response in oxidatively stressed U87-MG glioblastoma cells

Yesim Kaya

Muğla Sıtkı Koçman University, Muğla, TÜRKİYE

Oxidative stress is widely implicated in neurodegeneration and cancer biology, where reactive oxygen species generate DNA lesions and activate the ATR–CHK1 DNA damage response (DDR) to preserve genome integrity. Speedy/RINGO (SPY1) is an atypical cell-cycle regulator that can activate cyclin-dependent kinases through non-canonical mechanisms and has been linked to enhanced cell survival and anti-apoptotic effects across different cell types. However, how SPY1 intersects with DDR signaling—particularly under oxidative, degeneration-like stress—remains unclear. In this study U87-MG glioblastoma cells were transduced with SPY1/CD526-A lentiviral particles for 48 h and then exposed to 300 µM H_2O_2 for 24 h. Then, immunofluorescence was used to analyze the expression levels and subcellular distributions of SPY1, p-ATR, and p-CHK1, as well as the colocalization of SPY1 with p-ATR and p-CHK1. In U87-MG cells, oxidative stress and SPY1 expression produced distinct DNA damage response patterns. While p-ATR signals changed only slightly across conditions, p-CHK1 was more clearly influenced by SPY1, including under oxidative stress. Colocalization analysis further indicated increased overlap between SPY1 and p-CHK1, whereas SPY1/p-ATR overlap was reduced during oxidative stress. Together, these findings suggest that Speedy/RINGO biases DNA damage signaling toward a CHK1-linked checkpoint response rather than broadly enhancing ATR activation, supporting further studies on repair capacity and cell survival.

20. Systematic identification of DNA damage response dependencies in glioblastoma using a targeted CRISPR-Cas9 library

Ipek Kok[1,2,#], Canan Bayraktar[1,2,#], Altar Özbıyık[1,2], Alisan Kayabolen[1,2], Ozlem Yedier-Bayram[1,2], Ali Cenk Aksu[1,2], Ilknur Sur-Erdem[1,2], Tugba Bagci-Onder[1,2,*]

[1]Koç University School of Medicine, Brain Cancer Research and Therapy Laboratory, Istanbul, TÜRKİYE
[2]Koç University Research Center for Translational Medicine, Istanbul, TÜRKİYE
#Contributed equally
*Correspondence: tuonder@ku.edu.tr

Glioblastoma (GBM) exhibits extensive genomic instability and constitutive activation of DNA damage response (DDR) pathways that sustain tumor growth and therapeutic resistance. Systematic identification of DDR components essential for GBM viability may reveal actionable vulnerabilities. To this end, we generated a focused CRISPR/Cas9 sgRNA library, DDRKOL (DNA Damage Response Knock-Out Library), comprising 9,000 sgRNAs targeting 888 DDR-related genes (10 sgRNAs per gene) across homologous recombination, non-homologous end joining, replication stress signaling, DNA damage checkpoints, and cell cycle regulation. The library incorporates non-targeting guides and validated pan-essential genes for internal normalization and quality control.

Pooled loss-of-function screens were performed in U87-MG and A172 GBM cells. High concordance between biological replicates and consistent depletion of positive controls confirmed robust library performance and screening sensitivity. Integration with TCGA transcriptomic datasets demonstrated significant overexpression of prioritized candidates in GBM tumors relative to normal brain tissue, while DepMap analyses supported their conserved essentiality across cancer models.

Comparative analysis identified a shared subset of survival regulators, including TOP2A, XRCC6, RAD21, and CDK1. Individual gene disruption markedly reduced clonogenic survival and metabolic viability in established GBM lines, patient-derived primary cultures, and temozolomide-resistant derivatives. Competitive assays confirmed sustained negative selection of knockout populations. Mechanistically, depletion of these DDR hubs induced apoptosis and pronounced G2/M cell cycle arrest. In orthotopic intracranial xenograft models, TOP2A loss significantly impaired tumor progression and prolonged overall survival.

Collectively, these findings delineate core DDR-dependent survival networks in GBM and establish DDRKOL as a scalable platform to identify therapeutically targetable vulnerabilities.

21. Integrative single-cell multi-omics uncovers immune composition and immunosuppressive niches in paediatric high-grade gliomas

Anna M. Lenkiewicz[1], Bozena Kaminska[1], Katarzyna B. Leszczynska[1,2], Maciej Florczyk[1], Monika Dzwigonska[1,2], Szymon Lipiec[1,2], Shrinath Dhanapal[1], Wieslawa Grajkowska[3], Katarzyna Kotulska[3], Joanna Trubicka[3], Bozenna Dembowska-Bagińska[3]

[1]Laboratory of Molecular Neurobiology, Nencki Institute of Experimental Biology Polish Academy of Sciences, 02-093, Warsaw, POLAND
[2]Laboratory of Tumour Hypoxia and Epigenomics, Nencki Institute of Experimental Biology Polish Academy of Sciences, 02-093, Warsaw, POLAND
[3]The Children's Memorial Health Institute, 04-730 Warsaw, POLAND

Paediatric high-grade gliomas (pHGGs) are highly aggressive brain tumours and remain the leading cause of cancer-related mortality in children. Their treatment represents a major challenge in paediatric oncology, largely due to an immunologically “cold” and strongly immunosuppressed tumour microenvironment (TME). Limited understanding of tumour–host interactions within the brain hampers the development of effective immunotherapeutic strategies. The HIT-GLIO project aims to comprehensively characterise the pHGG TME, identify novel immunological targets, and propose therapeutic strategies capable of improving outcomes for paediatric patients. We integrated single-cell RNA sequencing (scRNA-seq) with a panel of 40 immune markers with single-cell T-cell receptor (scTCR) profiling and advanced computational analyses to define immune cell composition, functional states, and spatial niches shaping the pHGG immune landscape. To date, we analysed approximately 180,000 immune cells derived from tumour biopsies and matched cerebrospinal fluid (CSF) samples from eight pHGGs, including DMGs.

We found that pHGG-associated microglia exhibit transcriptional programs linked to extracellular matrix remodelling and angiogenesis, while showing low expression of chemokines and cytokines. Tumour-associated monocytes display reduced inflammatory signalling, and macrophages demonstrate predominantly phagocytic and immunosuppressive phenotypes. Combined scRNA-seq and TCR profiling further revealed that T-cell phenotypic diversity and clonality in tumours and CSF are shaped by distinct patterns of TCR utilisation. Collectively, our findings demonstrate a microglia-dominated, low-inflammatory and poorly chemotactic immune microenvironment in pHGGs, which likely contributes to limited lymphocyte infiltration and reinforces immunosuppressive microglial and macrophage states. These insights provide a foundation for identifying novel immune-targeted therapeutic strategies aimed at overcoming immune exclusion in pHGGs.

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22. CRISPR-based discovery of targetable vulnerabilities that modulate radiation response in glioblastoma

Altar Özbıyık[1,2], Nareg Değirmenci[1,2], Tugba Bagci-Onder[2,3,*]

[1]Koç University Graduate School of Health Sciences, Istanbul, TÜRKİYE
[2]Koç University Research Center for Translational Medicine, Istanbul, TÜRKİYE
[3]Koç University School of Medicine, Istanbul, TÜRKİYE
*Correspondence: tuonder@ku.edu.tr

Cancer cells exploit multiple cellular pathways to survive radiotherapy, contributing to treatment failure and tumor recurrence in glioblastoma (GBM). Identifying druggable regulators of radiation response is critical for developing effective combination therapies and improving clinical outcomes. Functional CRISPR screening combined with radiotherapy provides a systematic approach to uncover genetic vulnerabilities that modulate radiosensitivity and resistance mechanisms.

We performed a druggable genome–focused CRISPR knockout (KO) screen to identify determinants of radiotherapy response in GBM. A172 glioblastoma cells stably expressing Cas9 were transduced with a pooled druggable genome sgRNA library and cultured for 14–15 population doublings to ensure stable gene disruption while maintaining library representation. Following selection, cells were divided into two experimental arms: irradiated (+IR) and non-irradiated control (−IR). After competitive growth under treatment conditions, cell pellets were collected for genomic DNA extraction. Integrated sgRNA cassettes were amplified, prepared for next-generation sequencing (NGS), and analyzed using the MAGeCK pipeline to quantify differential sgRNA enrichment and depletion between conditions.

Comparative analysis identified candidate genes whose loss selectively impaired survival following irradiation, indicating potential radiosensitizers, as well as genes conferring relative radioresistance.
Collectively, our findings demonstrate that functional CRISPR screening enables unbiased identification of modulators of radiation response in GBM and provides a rational framework for prioritizing targetable pathways for combination treatment strategies aimed at overcoming therapeutic resistance.

23. Interpretable deep learning and radiomics from quantitative MRI predict tumor burden and survival in a preclinical glioblastoma model

Sara Ortega-Espina, Ana Sampedro-Viana, Sabela Fernández-Rodicio, Marcos Bazarra-Barreiros, Teresa Abengoza, Pablo Hervella, **Ramón Iglesias-Rey***

Neuroimaging and Biotechnology Laboratory (noBEL), Clinical Neurosciences Research Laboratory (LINC), Health Research Institute of Santiago de Compostela (IDIS), Santiago de Compostela, 15706, A Coruña, SPAIN
*Email: ramon.iglesias.rey@sergas.es

Background: Radiomics enables non-invasive quantification of tumor heterogeneity through high-throughput imaging feature extraction, yet its application in preclinical neuro-oncology remains underexplored. This study aimed to develop and validate an integrated radiomics and interpretable deep learning pipeline for early prediction of tumor volume and survival in a rodent glioblastoma model using longitudinal MRI.
Methods: Twenty-nine rats with orthotopic glioblastomas were distributed across two cohorts and imaged at 9.4 T on days 7, 14, and 21 post-implantation. A standardized five-step workflow was implemented: image acquisition, manual tumor segmentation, preprocessing (including denoising), extraction of 107 radiomic features, and predictive modeling. The TabNet deep learning architecture was benchmarked against conventional machine learning models (XGBoost, Random Forest, Support Vector Regression). Performance was evaluated via R^2, mean squared error, Bland–Altman analysis, and Spearman's correlation, with body weight incorporated as a biological covariate.
Results: TabNet consistently outperformed traditional models, achieving $R^2 > 0.85$ for tumor volume prediction and up to 0.92 for survival forecasting. Key discriminative features included morphological parameters (Least Axis Length, Maximum 2D Diameter Column, Mesh Volume) and gray-level dependence matrix metrics. Preprocessing strategy critically influenced performance: denoising enhanced survival prediction at advanced disease stages but offered limited benefit for early volume estimation. Notably, inclusion of body weight substantially improved volume prediction accuracy (R^2 increased from 0.740 to 0.926).
Conclusions: Quantitative MRI-based radiomics coupled with interpretable deep learning provides a robust, non-invasive biomarker for preclinical glioblastoma characterization. This approach facilitates animal stratification, treatment response monitoring, and predictive modeling, thereby strengthening translational neuro-oncology research. The dataset is publicly available at https://github.com/noBEL-MRI/Radiomics-Rat-Dataset.

24. Potential oncogenic role of PRSS1 identified by whole exome sequencing in glioma primary cell lines

Alvaro Monago Sanchez[1], Natalia Iglesias Bueno[2], Josefa Carrión Navarro[1,2], Laura Mateos Madrigal[1], Claudia Conquero Rodríguez[1], Sandra Fernández Martos[1], Ana Hernández Martinez[1], Cynthia Garcia de Jesus[3], Jorge Plazas[3], Claudia Quezada[4,5], **Noemi García-Romero[1,*]**, Angel Ayuso-Sacido[1,2,6*]

[1]Faculty of Experimental Sciences, Universidad Francisco de Vitoria, Madrid, SPAIN
[2]Brain Tumor Laboratory, Fundación Vithas, Grupo Hospitales Vithas, SPAIN
[3]Higher Polytechnic School, Universidad Francisco de Vitoria, Madrid, SPAIN
[4]Laboratorio de Biología Tumoral, Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile, Valdivia 5090000, CHILE
[5]Millennium Institute on Immunology and ImmuNOtherapy, Facultad de Ciencias, Universidad Austral de Chile, Valdivia 5110566, CHILE
[6]Faculty of Medicine, Universidad Francisco de Vitoria, Madrid, SPAIN

Background: Glioblastoma (GBM) is the most common and aggressive adult primary brain malignancy, with poor survival and marked resistance to therapy. In pediatric patients, diffuse midline gliomas (DMG), including diffuse intrinsic pontine gliomas (DIPG) with H3K27 alterations, are similarly lethal and completely refractory to therapy. These tumors present high inter and intratumoral heterogeneity, driven in part by glioma stemlike cells (GSCs), which compromises therapeutic responses and complicates model development. Whole-exome sequencing (WES) of patient derived GSCs can clarify subtype specific Single Nucleotide Variant (SNV)/Copy Number Variation (CNV) patterns and potentially expose novel vulnerabilities.
Methods: To identify recurrent molecular patterns and candidate therapeutic targets, we established a cohort of GSCs derived from eight patients, comprising six adult GBM and two pediatric DIPG, including H3 mutant and wild-type subtypes. WES of this cohort was performed to characterize copy number alterations and somatic point mutations.
Results: Adult-derived GBM IDH-Wild-Type GSCs recapitulated canonical genomic alterations including chromosome 7 duplication, chromosome 10 loss, EGFR amplification, and CDKN2A/B deletion. DIPG derived lines exhibited more heterogeneous genomic profiles, reflecting subtype-specific divergence. Importantly, all cell lines harbored a recurrent nonsense mutation in PRSS1 (p.Gly177*), truncating the catalytic domain. This mutation was also confirmed in a small cohort of GBM patients. Reduced PRSS1 expression correlated with poorer survival in GBM datasets, and we propose a mechanism whereby PRSS1 loss disrupts PAR2 signaling and promotes compensatory PAR1 activation, enhancing tumor progression.
Conclusions: Our study presents a comprehensive WES analysis of patient-derived GSCs, revealing key genomic alterations across adult and pediatric tumors. We identified a recurrent PRSS1 stop-gain mutation across all cells, suggesting a potential novel oncogenic role in gliomas of the PAR2 protease signaling axis, uncovering a common vulnerability and a potential therapeutic target in high-grade gliomas.

25. Synthetic genetic tracing of molecular and cellular heterogeneity in glioblastoma

M.J. Schmitt[1], C. Company[1], Y. Dramaretska[1], M. Serresi[1], B. Jiang[1], S. Kertalli[1], M. Grossmann[1], A. Aguzzi[2], I. Barozzi[3], G. Gargiulo[1]

[1]Max-Delbrueck-Center for Molecular Medicine, Molecular Oncology, Berlin, GERMANY
[2]University Hospital Zurich, Institute of Neuropathology, Zurich, SWITZERLAND
[3]Medical University of Vienna, Center for Cancer Research, Vienna, AUSTRIA

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.

Using our technology, 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 and 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 synthetic transcriptional reporters 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.

Keywords: Glioblastoma, Tumor Heterogeneity, Genetic Tracing, Tumor Microenvironment

26. High confidence context aware circRNA–miRNA–gene regulatory network in glioma derived from an LLM assisted knowledge graph

Mengyuan Wang

Teesside University, Middlesbrough, UK

Glioblastoma is characterised by profound regulatory complexity, yet existing circRNA studies in understanding glioma progression remain fragmented and difficult to integrate across biological contexts. Here, we present a biologically constrained, LLM-assisted framework for constructing high-confidence circRNA-centred regulatory knowledge graphs in glioma from large-scale literature. Applied to a pilot set of 500 glioma-related publications, the framework generated 959 unique entities and identified 80 studies containing high-confidence regulatory relationships (mean confidence = 0.848). The resulting network was dominated by canonical circRNA–miRNA–gene interactions, with sponging and miRNA-targeting relationships forming the principal regulatory backbone. Network-level analysis revealed convergence of multiple circRNA axes onto shared downstream effectors, including CCNB1 and FNDC1, suggesting coordinated oncogenic regulatory programs and potential hub structures. Among the identified pathways, the circNRIP1–miR-106a-5p–GPR133 axis emerged as a representative ceRNA cascade supported by high-confidence same-study evidence. Together, these findings demonstrate that biologically constrained LLM-assisted knowledge graph construction can recover mechanistically interpretable circRNA regulatory architecture while retaining critical contextual information. This framework provides a scalable foundation for high-resolution reconstruction of glioma regulatory networks and future AI-driven discovery of non-coding RNA biomarkers and therapeutic targets.

27. Functional genomics uncovers SFPQ-associated cell state regulation in IDH-mutant glioma

Ebru Yilmaz[1,2,3,4], Aybala Samancioglu[1,2], Alexander Jucht[3,4], Sedef Sarac Yalcinkaya[1,2], Mario L. Suvà[3,4], Tugba Bagci-Onder[1,2]

[1]Koç University Research Center for Translational Medicine (KUTTAM), Istanbul, TÜRKİYE
[2]Koç University School of Medicine (KUSOM), Istanbul, TÜRKİYE
[3]Department of Pathology and Krantz Family Center for Cancer Research, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA
[4]Gene Regulation Observatory, Broad Institute of MIT and Harvard, Cambridge, MA, USA

Gliomas are the most common central nervous system tumors in adults and are characterized by profound intra-tumoral heterogeneity. Their classification is based on histopathological features, molecular genetic alterations, and tumor aggressiveness. Among these, isocitrate dehydrogenase (IDH) mutations are frequently observed and are now established as key markers in CNS tumor classification. Although mutant IDH inhibitors are the center of therapeutic strategies, the identification of epigenetic vulnerabilities may reveal additional and complementary therapeutic intervention points.

To systematically uncover epigenetic regulators essential for IDH-mutant glioma viability, we performed a pooled CRISPR/Cas9-based epigenetic knockout screen in patient-derived IDH-mutant gliomasphere models. This screen identified SFPQ (splicing factor proline- and glutamine-rich) as one of the most significantly depleted genes, suggesting its critical role in tumor cell survival in this context. To investigate the functional consequences of SFPQ loss, we performed bulk RNA sequencing following SFPQ knockout. Transcriptomic profiling revealed downregulation of synaptic organization, proneuronal, and neuronal gene programs, accompanied by a shift toward a mesenchymal-like state characterized by activation of TNFα–NF-κB and interferon signaling pathways. In vivo studies to further validate these findings are currently ongoing.

Collectively, these findings identify SFPQ as a previously unrecognized regulator of lineage state maintenance in IDH-mutant glioma and nominate SFPQ as a potential epigenetic vulnerability with therapeutic relevance.

POSTER GROUP 2

5th Floor Poster Area · June 3, 2026 · 10:50 – 11:50

1. Impact of cranial radiotherapy on the developing brain: An MEG study of brain neural networks in pediatric acute lymphoblastic leukemia survivors

Georgia Kioselaki[1], Charis Styliadis[1], **Alkinoos Athanasiou[1],** Anastasios Siountas[1], Maria Kourti2, Panagiotis D. Bamidis[1]

[1]Medical Physics and Digital Innovation Laboratory, Aristotle University of Thessaloniki, 54124, Thessaloniki, GREECE
[2]Pediatric & Adolescent Hematology Oncology Unit, 2nd Pediatric Department, Faculty of Health Sciences, Aristotle University of Thessaloniki, AHEPA Hospital, 54124 Thessaloniki, GREECE

Background: Long-term survivors (LTSs) of pediatric acute lymphoblastic leukemia (ALL) are at increased risk of persistent neurocognitive impairments, particularly following exposure to prophylactic cranial radiotherapy (RT). While chemotherapy (CT) is associated with relatively mild cognitive effects, RT has been linked to more pronounced deficits affecting memory and attention. However, the long-term impact of RT on functional brain network organization remains insufficiently understood.
Objective: This study protocol aims to investigate treatment-related alterations in resting-state and task-based brain FC in LTSs of pediatric ALL and to examine their associations with cognitive performance.
Methods: This observational, cross-sectional study will include 80 participants distributed into three groups: 20 LTSs of pediatric ALL treated with CT and RT, 20 LTSs of pediatric ALL treated with CT only, and 40 healthy controls. All participants will undergo a single assessment session including resting-state and task-based magnetoencephalography (MEG), structural magnetic resonance imaging (MRI) for anatomical coregistration and assessment of changes in brain structure characteristics, and standardized neurocognitive testing. MEG data will be analyzed at the source level to estimate FC and brain network organization. Between-group differences and associations with cognitive performance will be examined using appropriate statistical analyses.
Conclusions: This study protocol aims to clarify the long-term effects of cranial RT on brain FC and cognitive performance in LTSs of pediatric ALL. The findings are expected to improve understanding of treatment-related neurotoxicity and to inform future follow-up and neurocognitive monitoring practices in this population and may also be relevant to other pediatric and adult populations receiving cranial radiotherapy, including patients treated for brain tumors.

2. Hydrogel-based therapeutic strategy to stimulate immune cell infiltration and remodel the glioblastoma microenvironment

Camille Cochois[1,2], Lucie Martin[1,2], Martine Dubois[1,2], Martin Pedard[1,2], Luc Picton[3], Cyrille Sabot[4], Virginie Dulong[3], Morgane Morel[3], Mathieu Madau[3], Pierrick Gandolfo[1,2], et Hélène Castel[1,2, 5]

[1]Inserm U1245, Cancer Brain Genomics (CBG), team NeuroGlio, 76000 Rouen, FRANCE
[2]IRIB, Institute for Research and Innovation in Biomedicine, Rouen, FRANCE
[3]UMR CNRS 6270, Polymers Biopolymers Surfaces, 76000 Rouen, FRANCE
[4]COBRA UMR 6014, FR 3038, INSA Rouen, CNRS, 76000 Rouen, FRANCE
[5]Cancer and Cognition Platform, 14000 Caen, FRANCE

Glioblastoma (GB) is a rare tumor of very poor prognosis. Failure in curative strategy is mainly due to a hypoxic and immunosuppressive microenvironment. Our aim is to tackle GB cells through a new angle and remodel the vascular and immune microenvironment. We thus built a hydrogel-based strategy engrafted with a chemokine to chemoattraction of GB cells, promote an anti-tumor response and favor cognitive rehabilitation.

To validate this strategy, a preclinical mouse model of GB, followed by a resection and injection of an HA-hydrogel grafted by an innovative fluorescent probe providing a dequenched signal in contact with invasive cells, showed that GB cells, macrophages, and endothelial vascular cells invade the hydrogel in the absence or the presence of a chemoattracttactant chemokine. Then, the therapeutics properties of the chemokine were studied through intratumoral injection or via the intracavity injection of the HA-hydrogel containing or not the chemokine. In both strategies, the chemokine led to a decreased tumor volume and increased immune cells infiltration, especially CD20+ B cells and CD8+ cytotoxic T cells through MECA76+ HEV immature structures, and prevented death of mice. Cognitive functions were evaluated through several behavioral tests before and after tumoral injection or resection-hydrogel chemokine treatment. We established that tumor is associated with « depression-like » behavior, sensorimotor deficits and impaired long-term memory. When injected or added in the hydrogel, the chemokine enabled recovery of cognitive functions.

This promising strategy based on a locally attraction of B cells and cytotoxic T cells allows the microenvironment remodeling and thus represents a new treatment opportunity for GB patients.

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3. HistoNeRF: A novel deep learning approach for 3D reconstruction of glioblastoma microarchitecture from standard 2D histology slides

Kubilay Doğan Kılıç

Ege University, İzmir, TÜRKİYE

Introduction: Glioblastoma (GBM) is characterized by high cellular heterogeneity and aggressive vascular proliferation. While histopathological analysis remains the gold standard for diagnosis and grading, traditional stained slides provide only two-dimensional (2D) information. This limitation hinders the comprehensive evaluation of complex 3D structures such as vascular tortuosity and tumor invasion fronts. Traditional 3D visualization methods, such as tissue clearing or manual reconstruction from serial sections, are time-consuming and labor-intensive, and prone to alignment artifacts. In this work, we propose HistoNeRF, a Neural Radiance Fields (NeRF)-based deep learning framework to synthesize volumetric 3D representations of GBM tissue directly from standard 2D histological images.
Methods: We used a dataset consisting of digitized serial sections of glioblastoma tissue. The HistoNeRF architecture is designed to learn a continuous volumetric scene function from a sparse set of 2D input views. Unlike traditional photogrammetry, NeRF optimizes a continuous coordinate function to estimate RGB color and volume intensity. We adapted this computer vision technique to microscopy by treating serial Whole Slide Images patches as camera views moving along the z-axis of the tissue block.
Results: Our preliminary results show that the HistoNeRF model can successfully interpolate between histological sections, generating new images and consistent 3D microarchitectural models. The model effectively highlights the continuity of microvascular structures, which are often misinterpreted in single 2D sections, and the spatial distribution of tumor cell clusters. This approach enables digital slicing of tissue at any angle and depth without physical re-cutting.
Conclusion: HistoNeRF is a significant step towards Computational 3D Pathology. By extracting 3D spatial information from traditional 2D slides without requiring specialized hardware or complex tissue clearing protocols, this tool offers a cost-effective solution for advanced tumor profiling. This aligns with the goal of integrating AI optimization into cancer research and potentially offers new morphological biomarkers for patient classification and prognosis in brain cancer.

Keywords: Glioblastoma, Neural Radiance Fields (NeRF), 3D Histology, Artificial Intelligence, Digital Pathology, Microvasculature.

4. Phosphoproteomics dissection of brain microenvironment-induced plasticity in cancer stem cells

Maruan Hijazi-Vega

Institute for Neurosciences of Castilla y Léon (INCyL), Salamanca, SPAIN
Department of Biochemistry and Molecular Biology. GIR Group “Bases Moleculares del Desarrollo”, University of Salamanca, SPAIN

Brain metastasis (BrM) remains a critical oncological challenge, characterized by a dismal prognosis and profound therapy resistance. This progression is driven by cancer stem cells (CSCs) which possess the unique capacity for tumor initiation and phenotypic adaptation. Within the brain, the metastatic niche imposes a distinct selective pressure through specialized glial populations, the blood-brain barrier, and unique metabolic constraints. However, the signaling cascades governing the bidirectional crosstalk between the neural microenvironment and CSCs remain poorly understood. In this study, we employ a high-resolution phosphoproteomic pipeline to elucidate the molecular reprogramming of CSCs during brain colonization. Using immunocompetent in vivo models of BrM, we demonstrate that the brain microenvironment significantly modulates the CSC phosphoproteome, triggering pathways essential for cellular plasticity and survival. Preliminary data reveal that niche-specific signals induce a fundamental shift in the signaling architecture of infiltrating tumor cells. By mapping these dysregulated kinase-substrate networks, we aim to identify the kinases responsible for maintaining CSC stemness within the brain parenchyma. This mechanistic insight is crucial for transitioning from non-specific treatments to targeted therapeutic strategies. Our findings provide a robust molecular framework to understand metastatic evolution, ultimately seeking to identify novel therapeutic vulnerabilities that sensitize resistant lesions and improve overall survival for patients with brain metastasis.

5. Systematic optimization of tumor-targeted VLP production and targeted protein delivery deadouts

Yasemin Holly Özoran[1,2], Canan Bayraktar-Odabaş[1,2], İbrahim Şahin Özyıldız[1,2], Tugba Bagci-Onder[1,2,3]

[1]Koç University Graduate School of Health Sciences, Medicine, Istanbul, TÜRKİYE
[2]Koç University Research Center For Translational Medicine, Istanbul, TÜRKİYE
[3]Koç University School of Medicine, Istanbul, TÜRKİYE

Virus-like particles (VLPs) offer a non-replicative platform for targeted protein transfer without genomic integration. EGFRvIII is a tumor-specific mutant receptor frequently expressed in glioblastoma and represents a promising target for selective therapeutic delivery. In this study, we focused on the systematic optimization of EGFRvIII-targeted VLP production and downstream analytical workflows to establish reliable experimental conditions for future therapeutic applications.

To optimize functional readouts during VLP production, a Gag-GFP fusion construct was employed as a reporter system. GFP directly fused to Gag enabled tracking of packaged protein delivery independently of plasmid-based expression systems. Flow cytometry was performed across multiple time points to characterize VLP delivery kinetics and degradation patterns. These analyses defined optimal temporal windows for flow-based detection and may inform future live-cell imaging approaches aimed at visualizing transient protein dynamics.

In parallel, VLP concentration methods were systematically compared. Polyethylene glycol (PEG)-mediated precipitation, protamine sulfate (PS)-mediated electrostatic aggregation, and ultracentrifugation were evaluated to improve VLP recovery and enhance detectable targeting efficiency. Flow cytometric analyses were used to assess the functional impact of each concentration strategy on GFP delivery.
Our findings demonstrate that both the concentration method and the analysis timing significantly influence measurable delivery outcomes. GFP kinetics confirmed the transient nature of VLP-mediated protein transfer, underscoring the importance of optimized detection windows.

As a prospective extension of these optimization efforts, immune activation and cytokine responses following VLP exposure may be evaluated to further refine characterization of this targeting platform.

Keywords: Virus-like particles (VLPs); Targeted protein delivery; Viral precipitation

6. Determining a role of transcription regulator Id1 in microglia induced by glioma using the CpG-Id1 siRNA

Yuliana Hovorova[1,2], Aleksandra Ellert-Miklaszewska[1], Bozena Kaminska[1]

[1]Laboratory of Molecular Neurobiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, 02-093, Warsaw, POLAND
[2]Department of Cryobiophysics, Institute for Problems of Cryobiology and Cryomedicine, National Academy of Science of Ukraine, 61016, Kharkiv, UKRAINE

Glioblastoma (GBM) is the most aggressive brain tumor in adults, with limited effectiveness of standard treatments such as surgery, radiotherapy, and chemotherapy. Resident microglia and infiltrating myeloid cells, which differentiate into immunosuppressive macrophages in the tumor microenvironment (TME), contribute to establishing an immunologically “cold” TME. Converting a “cold” to “hot” TME is a goal of immunotherapies. Myeloid cells are challenging to manipulate and various strategies have been explored to transport therapeutic agents to the TME and target immune cells. One of such tools is cytosine guanine dinucleotides (CpG) linked to DNA or siRNA that activate Toll-like Receptor 9 (TLR9) in myeloid cells, triggering inflammatory responses. We previously demonstrated that the transcription factor Id1 is upregulated in glioma-exposed myeloid cells and blocking Id1 expression with siRNA reverses their protumorigenic reprogramming while activating inflammatory genes (Ellert-Miklaszewska et al., Nanomedicine 2019). In this study we aim to evaluate a system for the targeted delivery of immunomodulatory siRNA to microglial cells to modify their immunosuppressive phenotype in microglia. We determined the efficacy of CpG-Id1-siRNA to inhibit Id1 expression in myeloid cells exposed to glioma in order to block their pro-tumoral reprograming and acquisition of immunosuppressive phenotype. We found that CpG-Id1-siRNA efficiently decreased target gene and protein expression in BV2 cells treated with GCM (glioma conditioned media) from GL261 glioma cells. Upregulation of pro-inflammatory and cytokine encoding genes such as Inos, Ccl5 and Il-1β was observed with increasing of concentration of CpG-Id1-siRNA. Altogether, we demonstrate that a newly designed CpG-Id1-siRNA targets myeloid cells and constitutes a promising tool for further investigations of its influence on activation of microglia-driven immunity. It could be a tool in anti-glioma therapies aiming at modifying the immunosuppressive TME.

7. Tumor mimics on brain MRI: Recognizing pseudotumoral intracranial lesions

Slavcho Ivanoski[1,2], Ognen Sheshoski[1,2], Mikjun Mikjunovikj[1]

[1]St. Erasmo Hospital for Orthopedic Surgery and Traumatology – Ohrid, NORTH MACEDONIA
[2]University St. Kliment Ohridski Bitola, NORTH MACEDONIA

Development and increased use of brain MRI contributed to more frequent detection of intracranial incidental findings. Majority of the incidental brain MRI findings are benign and require no further investigation or follow-up, but in certain cases they can present diagnostic dilemma and mimic true brain tumors.

The aim of the poster is to review the most common incidental MRI findings that can mimic tumors; to explain the imaging characteristics that can differentiate true brain neoplasms from pseudotumors. MRI examples of common tumor mimickers include developmental vascular abnormalities, inflammatory and demyelinating lesions, developmental variants, and benign cystic lesions, such as tumefactive demyelination, developmental venous anomalies, and arachnoid cysts are included in the poster. Their MRI appearance and imaging characteristics are briefly explained.

Knowledge of most common benign, incidental findings and their major imaging characteristics is important. Their differentiation from true brain neoplasms is essential for avoiding unnecessary procedures, follow up, or unnecessary interventions.

8. Advanced 3D cell culture models and microfluidic lab-on-chip devices for glioblastoma therapy evaluation

Bilal Javed

School of Food Science and Environmental Health, Technological University Dublin, IRELAND
Physics to Life Sciences Research Hub, Technological University Dublin, IRELAND
NanoLab Research Center, Technological University Dublin, IRELAND

The research project focuses on utilizing advanced cell culture 3D models and microfluidic lab-on-a-chip devices for a comprehensive understanding and pre-clinical treatment evaluation of glioblastoma. The project combines two technologies: 3D cell culture models and lab-on-a-chip microfluidic devices to develop a predictive patient-specific model to evaluate the prospective clinical therapies. Traditional 2D cell cultures often fail to represent the complex tumor microenvironment found in glioblastoma patients accurately. 3D cell culture models, on the other hand, can mimic the 3D architecture and cell-to-cell interactions present in tumors, offering a more realistic platform for studying glioblastoma behavior and drug response. We have developed and utilized 3D cell culture models incorporating glioblastoma cells with other relevant cell types found in the brain tumor microenvironment, such as endothelial cells and immune cells. This approach allowed for a more holistic investigation of glioblastoma growth, invasion, and response to potential therapies.

We are further working to integrate lap-on-a-chip technology. Organ-on-a-chip technologies using patient-specific cells, represent a promising ex-vivo testing platform as they allow for controllable cell culture within an organotypic microarchitectural environment, providing a simple yet more physiologically relevant platform for drug screening than traditional cell culture/animal models. These microfluidic devices can recreate microfluidic environments within a chip, allowing for precise control over factors like flow, pressure, and nutrient delivery. By incorporating 3D glioblastoma cell cultures into lab-on-a-chip devices, we will create an even more sophisticated in vitro 3D model that closely mimics the physiological conditions experienced by glioblastoma cells within the brain.

9. PEG versus chitosan surface coating increases chrysin loading and cytotoxic efficacy of Fe3O4 nanoparticles in vitro

Aynura Karimova[1], Sabina Hajizada[2]

[1]Baku State University, Baku, AZERBEIJAN
[2]Medical Faculty Mannheim, University of Heidelberg, GERMANY

Background: Surface functionalization can critically influence drug loading, physicochemical stability, and biological activity of magnetic iron oxide nanocarriers.
Aim: We compared PEG and chitosan coatings for chrysin-loaded Fe3O4 nanoparticles and assessed loading efficiency and in vitro anticancer activity.
Methods: Coated Fe3O4 nanoparticles were prepared and loaded with chrysin at defined ratios; structure and loading were evaluated by FTIR, XRD, and UV-Vis. Cytotoxicity and apoptosis were tested in HCT-116 cells using MTT and Annexin V/PI assays.
Results: PEG-coated nanoparticles showed consistently higher loading efficiency (72–77%) than chitosan-coated nanoparticles (45–58%). In HCT-116 cells, IC50 at 24 h was 16.68 µg/mL; at 5 µg/mL, PEG-coated chrysin-loaded nanoparticles inhibited viability more strongly than chitosan-coated counterparts (p=0.0033). After 48 h, PEG-coated nanoparticles induced higher apoptosis (61.7%) versus chitosan-coated (36.8%).
Conclusions: PEG coating improves chrysin loading and enhances in vitro antitumor effects while maintaining structural regularity under increased drug loading. These findings support PEG-based magnetic nanocarriers as a stable platform for further evaluation in tumor-cell models.

10. Combined HDAC and DNMT inhibition by mocetinostat and zebularine promotes apoptosis in U87MG glioblastoma cells

Hacer Kaya Cakir[1,2], Onur Eroglu[1,2]

[1]Department of Molecular Biology and Genetics, Faculty of Science, Bilecik Seyh Edebali University, Bilecik, TÜRKİYE
[2]Biotechnology Research and Application Center, Bilecik Seyh Edebali University, Bilecik, TÜRKİYE

Glioblastoma (GBM) is the most common and highly aggressive primary brain tumor in adults. Epigenetic alterations play a central role in the pathobiology of GBM. Therefore, targeting epigenetic mechanisms has emerged as a promising strategy in glioblastoma treatment. Mocetinostat (MGCD0103) is an isotype-selective inhibitor of human histone deacetylases (HDACs), enzymes and zebularine, a cytidine analog, functions as a DNA methyltransferase (DNMT) inhibitor and has been extensively investigated in epigenetic and oncology research. In this study, we aimed to elucidate the molecular mechanisms underlying the combined effects of mocetinostat and zebularine in U87MG glioblastoma cells. Cell viability was assessed using the MTT assay. Cell migration capacity was evaluated by wound healing assay. Apoptotic cell death was analyzed through Hoechst staining and caspase-3/7 activity assay. In addition, protein expression levels of caspase-3, caspase-7, and caspase-9 were determined by immunoblotting. Our results demonstrated that the combined treatment with mocetinostat and zebularine significantly reduced cell viability and suppressed migratory capacity compared to single treatments. Moreover, the combination markedly induced apoptosis, accompanied by alterations in caspase-3, -7, and -9 protein expression levels. These findings suggest that the combined epigenetic targeting of HDACi and DNMTi exerts enhanced anti-tumor effects in glioblastoma cells by inhibiting proliferation and migration while activating apoptotic pathways.

11. Investigating the invasive characteristics of patient derived glioblastoma cells in zebrafish

Natalia Kostilek[1], Tejas Borkar[2], Omkar Vinchure[2], Jay-Gopalakrishnan[2], Nathalie Jurisch-Yaksi[1]

[1]Department of Clinical and Molecular Medicine, Norwegian University of Science and Technology, Erling Skjalgssons gate 1, 7491 Trondheim, NORWAY
[2]Institute of Human Genetics, University Hospital, Friedrich-Schiller-Universität Jena, 07740, Jena, GERMANY

Glioblastoma (GBM) is a highly aggressive and lethal primary brain tumor, with a median survival of less than 18 months post initial diagnosis. Patients diagnosed with GBM face a “one therapy for all” approach that fails to better the overall prognosis. Current treatment options consist of surgical resection, chemotherapy and radiotherapy that slow down disease progression but rarely offers full recovery. This approach is insufficient as GBM is highly heterogeneous from one patient to the next. Furthermore, GBM stem cells develop resistance towards the present treatment approach, can renew and invade healthy tissue. Consequently, recurrence cannot be avoided. Currently, no cure is available. To address these unique and patient-specific features we combine invasion-assays in organoids and zebrafish to predict invasion patterns. These enable us to determine the specific invasive behaviors of patient-derived cells, in-vitro and in-vivo. We have successfully established zebrafish xenografts, replicating the invasive behaviors of patient-derived cells, in vivo. Our results suggest that GBM cells engraft in the zebrafish brain, undergo mitosis and limited apoptosis. Furthermore, they interact with zebrafish tissue by recruiting microglia towards the tumor as well as stimulate angiogenesis. Data obtained from zebrafish xenografts also reveal distinct patient-specific patterns of cellular invasion. Certain tumor cells exhibit higher aggressiveness, spreading more extensively than cells derived from other patients. Characterizing these distinct behaviors brings us closer to advancing personalized diagnostics and targeted drug discovery. Our models also provide robust platforms for evaluating and identifying the most effective treatments tailored to each patient, thus offering a more patient-specific approach.

12. Elucidating the potential and mechanistic roles of the cordycepin molecule in overcoming TMZ-resistance in glioblastoma multiforme via experimental and computational Studies

Kaan Adacan[1], **Aslı Kutlu[1,*]**

[1]Molecular and Biology Department, Faculty of Engineering and Natural Science, İstinye University, Istanbul, TÜRKİYE
*asli.kutlu@istinye.edu.tr

Glioblastoma (GBM), the most aggressive and therapeutically refractory primary brain tumor in adults, has a median survival of merely 14–16 months despite multimodal interventions. The clinical efficacy of the standard of care—maximal safe resection followed by chemoradiotherapy with temozolomide (TMZ)—is severely limited by intrinsic or acquired chemoresistance. This resistance is fundamentally driven by O^6-methylguanine-DNA methyltransferase (MGMT)-mediated DNA repair and the compensatory upregulation of alternative repair pathways. Here, we investigate a novel combinatorial strategy using cordycepin (3'-deoxyadenosine) to overcome TMZ resistance by inducing multi-factorial adaptations that target these complementary resistance nodes. We hypothesize that this synergistic approach is particularly relevant for difficult-to-treat, MGMT-proficient GBM phenotypes. To validate this, we integrated in vitro assessments using 2D and 3D cell culture models (including T98G) with advanced computational strategies. Through transcriptome profiling, molecular modeling, dynamics simulations, and binding energy calculations, we elucidate the mechanistic rationale underlying the Cordycepin-TMZ combination. Ultimately, this study positions cordycepin as a promising adjunctive candidate to reverse TMZ chemoresistance in GBM.

13. New approaches to biosensing

Bruno Mansano

University of Chemistry and Technology, Prague, CZECH REPUBLIC

Early detection of disease biomarkers in bodily fluids is essential for improving patient outcomes and reducing the physical and financial burden associated with advanced-stage treatment. Among emerging cancer biomarkers, galectins - particularly types 1 and 3 - have been linked to tumor progression and metastasis, including in brain cancers. However, current detection methods are often expensive, time-consuming, and require specialized laboratory procedures.

In this work, we present the development of a simple electrical biosensor designed to detect lectin-type proteins as models for galectins. The sensor is based on thin layers of tin oxide (Sno_2), a semiconducting material whose electrical properties change when specific biomolecules bind to its surface. To ensure selective detection, the surface was chemically modified with protein ligands that enable targeted recognition of the biomarker.

We carefully evaluated the material's stability and performance in aqueous and biologically relevant environments to ensure suitability for real-world applications. The resulting prototypes demonstrate reliable and selective detection through measurable electrical signals, even in complex biological media.

These findings highlight the potential of this technology as a rapid, cost-effective, and scalable platform for biomarker detection. With further development, such electrically readable sensors could contribute to earlier and more accessible cancer diagnostics in clinical practice.

14. Design and biological evaluation of chlorophenyl- and chlorophenoxy-substituted azole derivatives with enzyme modulation activity against neurodegeneration-related targets

Sam Dawbaa[1,2,3], **Demokrat Nuha[1,4,*]**, Asaf Evrim Evren[1], Begüm Nurpelin Sağlik[1], Leyla Yurttaş[1]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Anadolu University, Eskişehir, TÜRKİYE
[2]Department of Pharmacy, Faculty of Medical Sciences, Thamar University, Dhamar, YEMEN
[3]Department of Pharmacy, Faculty of Medical Sciences, Al-Hikma University, Dhamar, YEMEN
[4]Faculty of Medical Biochemistry, University for Business and Technology, Prishtina, KOSOVO
* Demokratnuha@gmail.com, demokrat.nuha@ubt-uni.net

In the present study, a series of newly developed azole-based compounds incorporating chlorophenyl and chlorophenoxy moieties were investigated for their enzyme modulation potential in the context of neurodegenerative diseases. The synthesized library comprised two distinct structural classes: hydrazide-linked thiazole derivatives (4a–4f) and triazole–thioacetamide hybrids bearing a benzothiazole core (6a–6c). These compounds were systematically evaluated for their inhibitory effects on acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and monoamine oxidase isoforms A and B (MAO-A and MAO-B), which are well-established molecular targets in the treatment strategies of Alzheimer's and Parkinson's diseases. Biological screening revealed that several derivatives exerted notable inhibitory activity. In particular, compounds 4e, 6a, and 6b emerged as the most effective AChE inhibitors, an observation that can be correlated with the presence of structural motifs known to favor cholinesterase binding. Regarding monoamine oxidase inhibition, compounds 6a and 6b displayed pronounced selectivity toward MAO-A, while derivatives 4a, 4d, 4f, and 6c demonstrated MAO-A inhibition exceeding 55%. Significant MAO-B inhibition was also recorded, especially for compounds 4a, 4f, 6a, and 6b, underscoring the importance of the azole-linked aryl ether framework in regulating the activity of both MAO isoforms. To further rationalize the observed biological behavior, density functional theory (DFT) calculations were performed, offering insight into the electronic characteristics of the studied molecules. Differences in HOMO–LUMO energy gaps, global reactivity descriptors, and charge distribution profiles were consistent with variations in enzymatic inhibition. Among the most potent candidates, compound 4a exhibited electronic features indicative of enhanced stability, whereas compounds 4f and 6a showed electronic environments favoring nucleophilic and electronegative interactions, which may contribute to their enzyme-binding capabilities. Molecular docking and binding-mode investigations revealed that the benzothiazole-containing derivatives preferentially oriented toward peripheral regions of the MAO active site, while the chlorophenyl and phenoxy substituents were positioned in proximity to the FAD cofactor within the catalytic cavity. Additionally, the acyl functionalities present in both series played a key role in stabilizing ligand–enzyme complexes through hydrogen-bond interactions with catalytically important amino acid residues, in agreement with previously reported binding modes of azole-based inhibitors.

15. Digital 'translational blindness' in neuro-oncology: Computational analysis of glioma clinical trial metadata

Iryna Oliynyk[1,2]

[1]Laboratory of Biochemistry of Animal Adaptation and Ontogenesis, Institute of Animal Biology NAAS, 38 V. Stus st., Lviv, 79034, UKRAINE
[2]Department of Physical and Chemical Disciplines, Lviv Medical University, 32 E. Paton st., Lviv, 79040, UKRAINE

Despite progress in computational methods for drug discovery, clinical translation in glioma treatment remains inefficient. One of the underestimated factors of this gap is the structural degradation of clinical registry metadata. This work systematically analyzed 2357 glioma clinical trial records from the WHO ICTRP registry as of January 2026. Using the developed Python-based pipeline, a critical asymmetry was detected: while the “study type” field has 100% occupancy, the data on the study phase demonstrate deep structural degradation. In particular, 550 records are completely devoid of information about the phase, and a significant part of the rest contains unstructured narrative descriptions, which makes them “invisible” to machine learning algorithms. The implemented indices — Reporting Gap (Gq) and Maturity Ratio (Mr) — confirmed the systemic gap between the early and late stages of translation. The analysis of Phase IV showed the dominance of optimizing existing standards instead of implementing new biological mechanisms. The results prove that semantic validation and unification of registries are a prerequisite for reliable integration of clinical data into artificial intelligence systems. Overcoming “translation blindness” requires moving from classical phase ontology to more flexible, machine-readable data models.

16. Virus-like particles crossing the blood-brain barrier: A platform for targeted glioblastoma delivery

İbrahim Şahin Özyıldız[1,2], Canan Bayraktar-Odabas[1,2], Yasemin Holly Ozoran[1,2], Tugba Bagci-Onder[2,3,*]

[1]Koç University Graduate School of Health Sciences, Istanbul, Türkiye
[2]Koç University Research Center for Translational Medicine, Istanbul, Türkiye
[3]Koç University School of Medicine, Istanbul, Türkiye
[*]Correspondence: tuonder@ku.edu.tr

Introduction: Glioblastoma is one of the most aggressive primary brain tumors, with therapeutic efficacy severely constrained by the blood-brain barrier (BBB). Virus-like particles (VLPs) are self-assembling nanostructures that mimic viral architecture without genetic material and allow surface functionalization, making them promising platforms for brain-directed delivery. In this study, we aim to establish a VLP-based system capable of crossing the BBB, providing a foundational platform for subsequent tumor-specific targeting.
Materials and Methods: To enhance receptor-mediated transcytosis, 13 BBB-shuttle peptides are being evaluated. Peptides are displayed on VLP surfaces, and particle characteristics are assessed by nanoparticle tracking analysis. Transport studies are performed using human and mouse in vitro BBB transwell models, including both endothelial monolayers and tri-culture systems composed of endothelial cells, astrocytes, and pericytes. Barrier integrity is validated by transendothelial electrical resistance (TEER) measurements and sodium fluorescein permeability assays. VLPs incorporate gag-fused GFP to enable fluorescence-based evaluation.
Results and Conclusion: Functional human and mouse BBB models were established in both monolayer and tri-culture configurations, with barrier integrity confirmed by TEER and sodium fluorescein permeability assays. Peptide-displaying VLPs were generated and characterized by nanoparticle tracking analysis. GFP incorporation was confirmed, and fluorescence measurements showed strong dose-dependent linearity (R^2 = 0.9907), supporting their suitability for transport studies. These findings provide a validated framework for future integration of glioblastoma-specific targeting strategies.

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17. Targeted degradation of pre-miR-21 in glioma via brain-permeable RIBOTACs

Alberto P.[1], Sdei F.[1], Bagnolini G.[1], Graziosi A.[1], Ghelli L.[1], Sosic A.[2], Fabbian S.[2], Duca M.[3], Uliassi E.[1], Bolognesi M.L.[*1]

[1]Department of Pharmacy and Biotechnology, University of Bologna, Via Belmeloro 6, 40126, Bologna, ITALY
[2]Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Via F. Marzolo 5, 35131 Padova, ITALY
[3]Université Côte d'Azur – CNRS, Institute of Chemistry of Nice, 28 avenue Valrose, 06100 Nice, FRANCE

We are witnessing a paradigm shift in drug discovery, with RNA emerging as a compelling therapeutic target beyond traditional protein-centric approaches. In brain cancer, RNA targeting enables direct modulation of oncogenic gene expression, providing access to previously undruggable drivers. A central regulator of glioma progression is miR-21, an oncomiR currently under investigation as a circulating biomarker for several solid tumors. (10.1016/j.omtn.2020.03.003).

Ribonuclease Targeting Chimeras (RIBOTACs) are an innovative class of small-molecule degraders that promote catalytic RNA degradation by recruiting RNase L through induced ternary complex formation (10.1038/s41586-023-06091-8). These heterobifunctional molecules consist of a target-binding ligand linked to an RNase L–recruiting moiety. Our goal is to develop brain-permeable RIBOTACs that selectively degrade pre-miR-21 to reduce miR-21 levels and suppress tumour growth.

Because achieving high affinity and selectivity for structured RNA remains a major challenge, we are designing pre-miR-21 binders guided by orthogonal structural evidence, integrating HR-MS, NMR, computational modelling, and biological evaluation. Several compounds exhibit nanomolar binding affinity and micromolar cellular activity, and the most promising candidates are being advanced into RIBOTACs.

18. Tumor subtype-specific reprogramming of microglial states in breast cancer brain metastasis

Patrycja Rosa[1], Anna M. Lenkiewicz[1], Chang Park[2], Lena Jassowicz[2], Christel Herold-Mende[2], Bozena Kaminska[1]

[1]Laboratory of Molecular Neurobiology, Nencki Institute, Warsaw, POLAND
[2]Division of Experimental Neurosurgery, University of Heidelberg, GERMANY

Brain metastases (BrMets) develop in the central nervous system (CNS) and are a clinical burden. Mechanisms behind tumor evoked changes within the metastatic niche remain poorly known. Understanding immune remodeling in brain metastasis requires high-resolution characterization of resident and infiltrating cell states. We performed CITE-seq profiling of >50,000 CD45+ cells from murine HER2+ and triple-negative breast cancer (TNBC) brain metastasis models to define subtype-specific immune ecosystems. Integrated transcriptomic and surface protein analysis resolved 24 immune cell types and delineated multiple microglial states, including homeostatic, activated, phagocytic, and proliferative subsets. Comparative studies of composition and differential expression revealed tumor subtype–dependent microglial transitions. HER2+ metastases preserved a predominately homeostatic microglial compartment, whereas TNBC metastases induced a coordinated shift toward inflammatory and proliferative states, alongside substantial neutrophil expansion. Pathway enrichment analyses further demonstrated subtype-specific activation of immune signaling and metabolic programs, suggesting distinct regulatory networks governing microglial plasticity. Our findings indicate that tumor subtype dictates the trajectory of microglial reprogramming in brain metastasis. Rather than a uniform immunosuppressive niche, BrMet comprises distinct, subtype-dependent immune ecosystems. These data provide a framework for targeting tumor–microglia interactions in a subtype-specific manner.

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19. Cold atmospheric plasma enhances cytotoxicity of liposome-encapsulated gold nanoparticles in glioblastoma cells

Malik Hamza Sajjad[1,2], Bilal Javed[1], Furong Tian[1,3], Paula Bourke[4]

[1]School of Food Science and Environmental Health, Technological University Dublin, Dublin, IRELAND
[2]Sustainability and Health Research Hub, Greenway Hub, Grangegorman, Dublin, IRELAND
[3]FOCAS Research Institute, Technological University Dublin, Dublin, IRELAND
[4]School of Biosystems and Food Engineering, University College Dublin, Dublin, IRELAND

Glioblastoma is the most aggressive form of brain cancer and remains difficult to treat due to its invasive nature and limitations in effective drug delivery. To address these challenges, novel therapeutic approaches such as nanoparticle-based delivery systems are being investigated. This study explores the combined use of liposome-encapsulated gold nanoparticles (AuNPs) and cold atmospheric plasma (CAP) to enhance cytotoxicity in glioblastoma cells. Liposomes improve nanoparticle stability and cellular uptake, while CAP has been shown to enhance intracellular delivery and induce oxidative stress. This study hypothesizes that the combination of liposome-encapsulated AuNPs and CAP will enhance glioblastoma cell death through ROS-mediated mechanisms. Encapsulation of the gold nanoparticles into the Dipalmitoylphosphatidylcholine (DPPC) and cholesterol liposomes (molar ratios 7:5) was found to decrease the IC50 value for gold nanoparticles from 27 µg/ml to 6.64 µg/ml in U-251 MG cells. Furthermore, the cells will be treated with liposome-encapsulated AuNPs with CAP exposure. After the treatment, cell viability and ROS production will be quantified. Additionally, the effects of varying DPPC-to-cholesterol ratios on the cytotoxicity will be evaluated. This study aims to investigate the role of reactive oxygen species (ROS) in the synergistic cytotoxicity induced by gold nanoparticles and cold atmospheric plasma, contributing to the development of more effective therapeutic strategies for glioblastoma.

20. Exploration of novel molecules against paediatric diffuse midline glioma

Álvaro Monago Sánchez[1], Laura Mateos Madrigal[1], Sandra Fernández Martos[1], Pepa Carrión Navarro[1], Noemi García Romero[1], Ángel Ayuso Sacido[2]

[1]NeuroOnco Lab, Facultad de Ciencias Experimentales, Universidad Francisco de Vitoria, Madrid, SPAIN
[2]Preclinical Models of Brain Tumors Lab (BTL), Unidad Funcional de Enfermedades Crónicas (UFIEC), Instituto de Salud Carlos III (ISCIII), Madrid, SPAIN

Paediatric diffuse midline glioma (DMG), is an aggressive, infiltrative tumour arising in midline structures and is associated with dismal outcomes, with radiotherapy as the only clinical option. A defining molecular feature in most cases is the H3K27M (H3K27-altered) histone mutation, which drives widespread epigenetic and transcriptional dysregulation and sustains tumour growth through stem-like cellular states. New therapeutic strategies that effectively disrupt these aberrant programmes are urgently needed.

Here, we established and characterised two patient-derived glioma stem-like cell (GSC) models from paediatric DMG: one H3K27M-mutant and one H3K27 wild-type. We evaluated four proprietary, undisclosed small molecules provided by an industry partner: three structurally related analogues designed to block aberrant transcriptional activity, and a fourth compound targeting microtubules.

All four compounds demonstrated reproducible anti-tumour activity in these GSC models, with greater in vitro efficacy than conventional comparator agents like TMZ tested in parallel. The transcription-targeting series induced marked shifts in transcriptional status and altered the composition of chromatin-bound proteins, consistent with disruption of oncogenic transcriptional control. The microtubule inhibitor produced strong cytotoxic effects consistent with mitotic disruption.

Collectively, these data identify multiple novel chemical entities with promising activity against DMG cellular models. While encouraging, further work in expanded patient-derived cohorts and additional preclinical models, including in vivo systems, is required to define therapeutic windows, mechanisms, and translational potential.

21. Gyrophoric acid enhances temozolomide-induced effects in glioblastoma cell lines

Patrik Šimko[1], Nicol Urbanska[1], Simona Mattova[1], Beata Leskova[2], Alzbeta Blicharova[1], Adam Nedoroscik[1], Marcela Capcarova[3], Terezia Kiskova-Simkova[1]

[1]Department of Pathology, Faculty of Medicine, Pavol Jozef Safarik University in Kosice, SLOVAKIA
[2]Department of Pharmacology, Faculty of Medicine, Pavol Jozef Safarik University in Kosice, SLOVAKIA
[3]Department of Animal Physiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, SLOVAKIA

Gyrophoric acid (GA), a lichen secondary metabolite, has attracted increasing attention due to its reported biological activities. In this study, we evaluated the antiproliferative and modulatory effects of GA alone and in combination with temozolomide (TMZ) in human glioblastoma cell lines U118, U251, and U87 using the MTT assay after 24 and 48 hours of exposure. GA alone exhibited moderate, dose- and time-dependent effects on cell viability. After 24 hours, cytotoxicity was limited and cell line-dependent, with U118 cells showing the highest sensitivity. Prolonged exposure (48 hours) resulted in a more pronounced reduction in viability, particularly at concentrations ≥300 µM. U251 cells were the most resistant, whereas U118 and U87 displayed increased vulnerability. Therefore, in further experiments, U87 cell line was selected. TMZ alone induced only a moderate reduction in viability at the tested time points in a dose dependent manner. However, combined treatment with GA (100 µM) and TMZ (100 µM) led to a greater decrease in cell viability showing synergic effect of both compounds, especially after 48 hours. These findings suggest that GA shows moderate intrinsic antiproliferative activity and may act as a chemosensitizing agent, enhancing temozolomide efficacy in a cell line-, dose-, and time-dependent manner. Further studies focusing on apoptosis, oxidative stress, and DNA repair pathways are needed to evaluate the underlying mechanisms of this interaction.

22. Pathology specific m6A RNA modifications in the brain

Daina Skiriute[1], Raulas Krusnauskas[2], Giedrius Steponaitis[1], Rugile Dragunaite[1], Rytis Stakaitis[1], Paulina Vaitkiene[2]

[1]Laboratory of Molecular Neurooncology, Neuroscience Institute, Medical Academy, Lithuanian University of Health Sciences, LITHUANIA
[2]Laboratory of Molecular Neurobiology, Neuroscience Institute, Medical Academy, Lithuanian University of Health Sciences, LITHUANIA

GBM is the most deadly and prevalent brain tumor, with 12-15 months median survival. High heterogeneity of the tumor makes therapeutic development and track of disease extremely challenging. Recent development of ONT direct RNA sequencing technologies enabled researchers to explore RNA modifications like N6-methyladenosine (m6A) and their value as novel biomarkers of the disease. M6A is the most frequent post-transcriptional RNA modification detected in ~25% of mRNA transcripts. M6A affects cell fate, differentiation, and multiple molecular pathways: splicing, mRNA stability, and localization. Multiple groups reported that m6A machinery consisting of “writers”, “readers” and “erasers” is deregulated and strongly associated with GBM progression. The aim of this work was to determine whether glioblastoma exhibits distinct m6A RNA modification profiles—both in frequency and in the positional distribution of m6A sites across gene transcripts. Here we used long-read Nanopore RNA sequencing technology to assess transcriptome wide m6A pattern in glioblastoma patient samples and healthy brain samples. Base calling was done with Guppy, and candidate m6A positions were detected with m6Anet. We looked at m6A candidate sites in differentially and constitutively expressed mRNAs to identify glioblastoma specific m6A mRNA modifications.

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23. Proteomic analysis of the in vitro anti-cancer effects of all-trans retinoic acid and curcumin combination therapy reveals molecular insights in U87 glioblastoma cells

Ceyda Sönmez[1], Büşra Ergün[1], Aleyna Baltacıoğlu[1], Aysel Özpınar[1,2*]

[1]Department of Biochemistry and Molecular Biology, Graduate School of Health Sciences, Acibadem Mehmet Ali Aydinlar University, Istanbul, 34752, TÜRKİYE
[2]Department of Medical Biochemistry, School of Medicine, Acibadem Mehmet Ali Aydinlar University, Istanbul 34752, TÜRKİYE
*Correspondence: aysel.ozpinar@acibadem.edu.tr

Background: Glioblastoma multiforme (GBM) is a highly aggressive brain tumor with poor prognosis, characterized by rapid progression, high recurrence, and resistance to conventional therapies. The limited efficacy of single-agent treatments in heterogeneous tumors has led to growing interest in combination therapies.
Aim: The goal of this study was to investigate the in vitro antitumoral effects of ATRA and CURC combination therapy in U87-MG glioblastoma cells and to explore the underlying molecular mechanisms through proteomic profiling.
Methods: Cell viability, migration and apoptosis assays were performed to evaluate the antitumoral effects of ATRA and Curcumin, both individually and in combination. U87-MG glioblastoma cells were treated with experimentally determined doses for 72 hours and cell pellets were stored at −80 °C. Pellets were lysed in a urea-based buffer containing protease and phosphatase inhibitors. Proteins were reduced with DTT and alkylated with chloroacetamide, followed by dilution and overnight tryptic digestion. The resulting peptides analyzed with nano-LC-MS/MS. Data were processed using MaxQuant and UniProt database search, followed by statistical evaluation with Perseus. Differentially expressed proteins were subjected to pathway enrichment analysis.
Results: The combination therapy demonstrated a synergistic effect, significantly reducing proliferation and migration compared to monotherapies. Proteomic analysis revealed substantial remodeling of signaling networks, including alterations in cell cycle regulation, protein synthesis, angiogenesis, invasion, DNA replication, and critical signaling pathways.
Conclusion: ATRA and curcumin combination stands out as a potent strategy with high therapeutic potential by simultaneously targeting multiple critical signaling pathways in GBM cells.

24. Molecular response of neural stem cells to glioblastoma-derived extracellular vesicles

Greta Žvinakytė[1,2], Onur Ates[1], Toma Dievaitė[1], Daina Skiriutė[1], Tomasz Slebioda[3], **Giedrius Steponaitis[1,*]**

[1]Laboratory of Molecular Neurooncology, Neuroscience Institute, Lithuanian University of Health Sciences, Kaunas, LITHUANIA
[2]Faculty of Medicine, Medical Academy, Lithuanian University of Health Sciences Lithuanian University of Health Sciences, Kaunas, LITHUANIA
[3]Department of Histology, Medical University of Gdansk, Gdansk, POLAND
*Correspondence: giedrius.steponaitis@lsmu.lt

Background: Glioblastoma (GB) progression is strongly shaped by interactions within the tumor microenvironment, particularly through extracellular vesicles (EVs) that mediate communication with surrounding cells. Because GBs often arise near the subventricular zone, a niche rich in neural stem cells (NSCs), tumor-derived signals may directly affect NSC behavior and potentially contribute to tumor development or recurrence. Despite this close spatial and functional relationship, the molecular responses of NSCs to GB-derived signals remain unclear. Therefore, this study investigated how single-dose exposure with GB derived EVs influence NSC transcriptomic changes.
Methods: EVs from LN229, U87-MG cells were isolated via 12% PEG precipitation and characterized by NTA, TEM, flow cytometry, and ELISA for CD63, HSP70, and APO-A1. NSC H9 cells were stimulated with GBM-derived EVs (~1250 EVs/cell) or control EVs for 24h, after which RNA was isolated for NGS.
Results: NSC H9 were stimulated with GB-derived EVs, followed by RNA-seq to evaluate their transcriptomic responses. RNA-seq detected ~40,000 expressed genes, from which a core set of shared and differentially expressed genes between the treated and control groups was established. Subsequently, the enrichment analysis revealed significantly altered GO terms. The highest enriched pathway was found to be related to cell migration and negative regulation of cell adhesion.
Conclusions: Ultimately, these findings demonstrate that even a single-dose stimulation with GBM-EVs successfully initiates significant pro-invasive transcriptomic reprogramming in NSCs, revealing the crucial early molecular events that drive altered cellular behavior.

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25. Standardizing patient-derived glioblastoma organoids: A high-fidelity biobanking platform to preserve intra- and intertumoral heterogeneity

Melis Ercelik[1], Cagla Tekin[1], Pınar Eser[2], Ahmet Bekar[2], Hasan Kocaeli[2], Mevlut Ozgur Taskapilioglu[3], **Berrin Tunca[1]***

[1]Department of Medical Biology, Faculty of Medicine, Bursa Uludag University, Bursa, TÜRKİYE
[2]Department of Neurosurgery, Faculty of Medicine, Bursa Uludag University, Bursa, TÜRKİYE
[3]Department of Neurosurgery, Faculty of Medicine, Istanbul Yeni Yuzyıl University, Istanbul, TÜRKİYE

Objective: Glioblastoma (GB) is characterized by a complex cellular landscape that contributes to therapeutic resistance and poor clinical outcomes. A critical challenge in neuro-oncology is the lack of preclinical models that accurately reflect this diversity. This study aims to establish a standardized and reproducible pipeline for generating patient-derived GB organoids (GBOs) that preserve the spatial and molecular heterogeneity of the parental tumors, thereby providing a robust platform for translational research and personalized drug testing.
Materials and Methods: Surgical specimens were obtained from patients diagnosed with GB. To bridge the gap between benchside research and clinical relevance, tissues were micro-dissected (0.5–1 mm) and cultured in a defined organoid medium. An orbital shaker-based system was utilized to ensure long-term viability and structural integrity. The fidelity of the GBOs was validated through a comparative immunohistochemical (IHC) panel—including GFAP, Olig2, IDH-1, p53, ATRX, and Ki67—analyzing the expression patterns before and after organoid formation to ensure the preservation of patient-specific signatures.
Results: The results demonstrated that the organoid models maintain the key histopathological and molecular hallmarks of the original tumors. The high success rate in establishing these cultures and their ability to mirror the intratumoral heterogeneity of the source material confirm the reliability of the protocol.
Conclusion: Our findings highlight patient-derived GB organoids as a superior, scalable model for investigating disease mechanisms and accelerating the development of personalized treatment strategies. By implementing a standardized biobanking approach, this model facilitates cross-disciplinary collaboration and provides a valuable resource for large-scale, multi-center brain cancer studies.

Keywords: Glioblastoma, Organoid culture, Tumor heterogeneity, Translational Oncology, Biobanking.

26. P53 hotspot variants differentially shape neurodevelopmental and oncogenic pathways in Li-Fraumeni brain organoids relevant to pediatric brain tumors

Yann Zerguine[1,2], Gabin Carpentier[1,2], Laurence Desrues[1,2], Jeanne Louis[1,2], Lucie Martin[1,2], Gaëlle Bougeard-Denoyelle[1,2], Claude Houdayer[1,2], Gaël Nicolas[1,2], Hélène Castel[1,2], Olivier Wurtz[1,2]

[1]INSERM U1245, Cancer and Brain Genomics, Université Rouen Normandie, Rouen, FRANCE
[2]Institut de Recherche et d'Innovation Biomédicale (IRIB), 76000 Rouen, FRANCE

Pediatric tumors differ substantially from adult counterparts, particularly through a stronger contribution of inherited predisposition. Germline TP53 variants underlie Li-Fraumeni syndrome (LFS), a disorder characterized by early-onset malignancies, broad tumor spectrum, and pronounced clinical heterogeneity. LFS pediatric brain tumors vary widely in location and type suggesting intersection between TP53 mutation class and neurodevelopmental programs. However, limited understanding of oncogenic mechanisms in LFS-associated gliomas hampers the discovery of early biomarkers and actionable targets.

To investigate how pathogenic TP53 variants influence early neurodevelopmental trajectories and oncogenic mechanisms, we modeled these processes using unguided cerebral organoids derived from human induced pluripotent stem (hiPS) cells engineered to carry LFS-associated 'hotspot' p53 mutations p.R248W and p.R337H.

Both variants induced early expansion of neural progenitor populations together with mutation-specific effects on neuronal differentiation. P53(R337H) produced the most pronounced phenotype, promoting marked increases in Sox2+ neural, Pax6+ radial glia progenitors and cortical SATB2+ neuron differentiation, whereas p53(R248W) primarily enhanced progenitor amplification. Across all genotypes, mutant organoids exhibited elevated genotoxic stress and genetic instability, evidenced by increased γH2AX+ double-strand breaks and enlarged nuclear size.

Collectively, these findings indicate that disruption of p53 function may create a permissive genomic context favoring acquisition of secondary oncogenic alterations. The dependence on both mutation type and allelic dosage supports a model in which variant-specific effects and loss of heterozygosity jointly shape the phenotypic variability observed in Li-Fraumeni syndrome. These results provide mechanistic insight into pediatric brain tumor susceptibility and highlight potential avenues for biomarker development and targeted therapeutic screening.

27. Crosstalk between glioblastoma and natural killer cells in 3D models

Pia Žižek[1,2,*], Špela Kladnik[1], Tina Kolenc Milavec[1,2], Anamarija Habič1[,2], Urban Švajger[3,4], Andrej Porčnik[5], Metka Novak[1,6], Barbara Breznik[1,7]

[1]Department of Genetic Toxicology and Cancer Biology, National Institute of Biology, Ljubljana, SLOVENIA
[2]Jožef Stefan Postgraduate School, Ljubljana, SLOVENIA
[3]Blood Transfusion Centre of Slovenia, Ljubljana, SLOVENIA
[4]Faculty of Pharmacy, University of Ljubljana, Ljubljana, SLOVENIA
[5]Department of Neurosurgery, University Medical Centre Ljubljana, Ljubljana, SLOVENIA
[6]Biotechnical Faculty, University of Ljubljana, Department of Biology, Ljubljana, SLOVENIA
[7]Faculty of Chemistry and Chemical Engineering, University of Ljubljana, Ljubljana, SLOVENIA
*E-mail: pia.zizek@nib.si

Glioblastoma (GBM) is a highly aggressive grade IV glioma and the most common primary tumour of the central nervous system in adults. Inefficient treatment is mostly due to heterogeneity and therapeutic resistance, latter driven by the complex tumour microenvironment (TME). There is still a gap in understanding GBM resistance to therapy in connection to TME, as well of relevant tumour models that mimic GBM-immune cell interactions, especially the dynamic component of the immune system.

To better understand GBM-NK cell interactions and study GBM-immune cell interactions after they enter the TME, we have established spheroids from differentiated (GB cells) or glioblastoma stem cells (GSC) and co-cultured them with NK-92 cells. To better mimic the in vivo conditions with the dynamic influx of immune cells into the tumour, a microfluidics platform (MIVO® platform, React4life) was set up, where spheroids were cultured in the tumour chamber above microcirculation of NK cells. To determine the infiltration of labelled NK cells into the spheroids and the viability of GBM and NK cells, flow cytometry, immunofluorescence and fluorescence microscopy were performed.

In the static model, NK cells more efficiently infiltrated GB cell spheroids but showed much higher cytotoxicity against GSC spheroids. As observed in the static model, NK cells infiltrated the GSC spheroids at lower levels. We observed no effect on infiltration of NK cells into spheroids in this setting due to effector:target ratio.

We have successfully established 3D models from GB and GSC cells. Furthermore, we have successfully established a microfluidic platform that mimics tumour-immune cell interactions in human patients. Our results show that GBM-NK crosstalk regulates NK cell activity. With these results we can further explore GBM biology and test new targeted therapy and NK based immunotherapy.