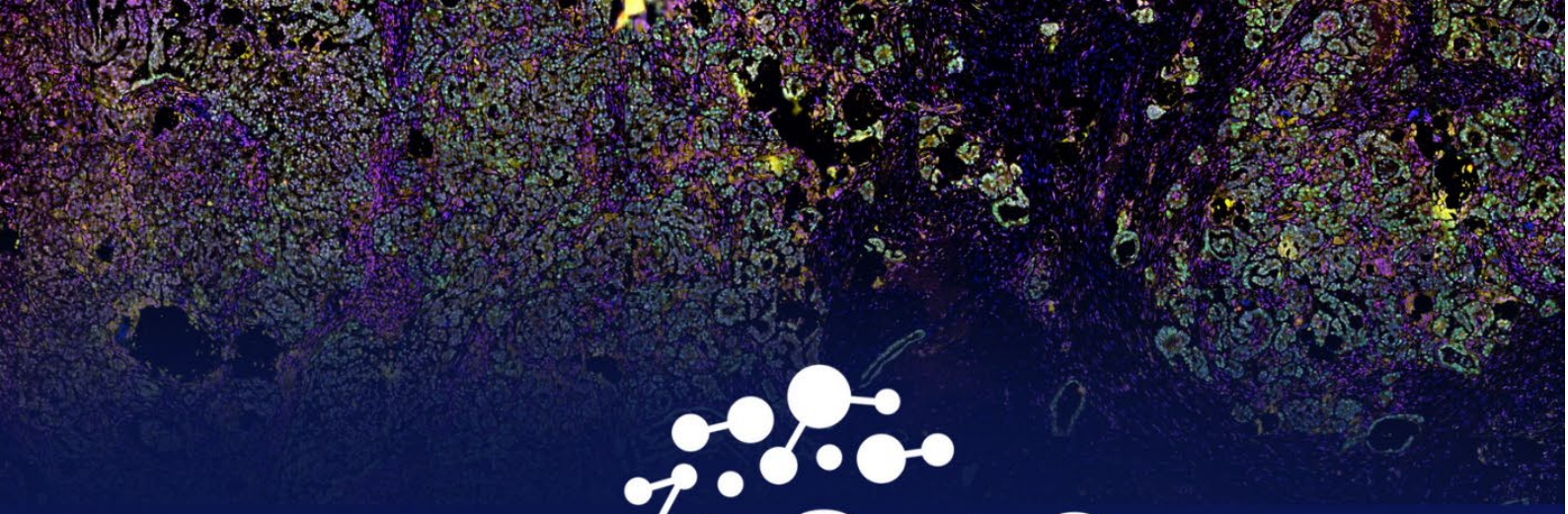

CutCancer final conference 2025

SHAPING TOMORROW: INNOVATIVE APPROACHES IN GENETIC TOXICOLOGY AND CANCER RESEARCH

October 22nd-23rd, 2025

National Institute of Biology (NIB)

BOOK OF ABSTRACTS

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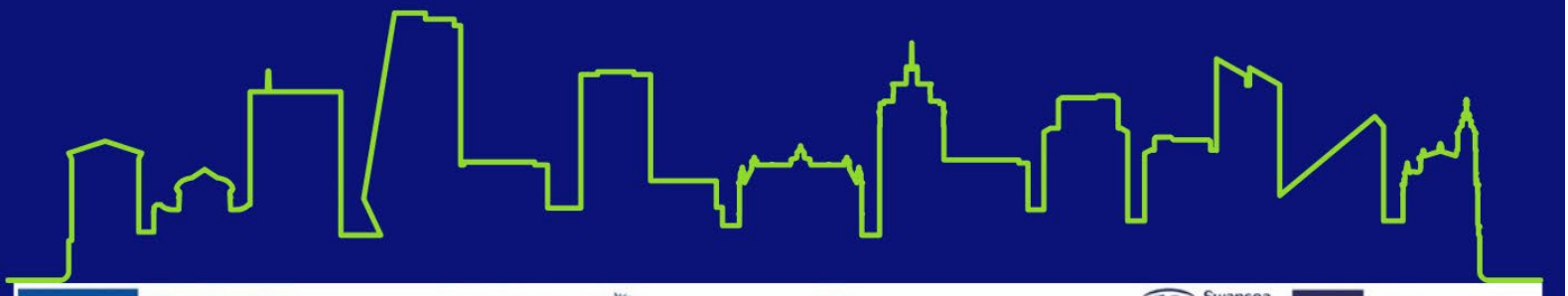

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- Chika Yokota, Stockholm University, Sweden

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Assoc. Prof. Bojana Žegura, PhD

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Abstracts of keynote lectures

1. The Importance of Multi-endpoint Approaches in the Development of New Approach Methodologies (NAMs) for Carcinogenicity

Gareth Jenkins[1]

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Genetic toxicology is a vital aspect in the safety assessment of new products, as DNA damage can drive carcinogenesis decades after sustained exposures. Traditional genotoxicity testing involves both cell-based (*in vitro*) and animal-based (*in vivo*) tests in most chemical sectors through a tiered approach. The European Union Cosmetics directive banned animal testing for cosmetic ingredients, and recent announcements by the FDA about phasing out animal testing for chemical safety have driven the need to develop new approach methodologies (NAMs) as alternatives to animal testing.

NAMs have actually been developed over recent decades as more sophisticated cell-based (and tissue-based) approaches designed to replace *in vivo* tests. We have been actively developing multi-endpoint approaches coupling DNA damage assessment to other cancer-relevant endpoints (cell cycle, apoptosis, ROS induction, cell morphology). We have developed a multi-endpoint suite of tests and validated this approach with many genotoxic carcinogens (as well as testing non-genotoxic carcinogens, which are traditionally negative in genotoxicity tests). However, there are still many questions unanswered relating to NAMs, including the balance between acute and chronic exposures, the optimal sampling time of the different endpoints and the optimal cell model (s) to use. We have explored many of these issues and believe that using optimal multi-endpoint approaches will revolutionize safety testing and remove animal testing over the coming years.

Our research group at Swansea University has been at the forefront of developing NAMs for genotoxicity testing, the future of regulatory testing is exciting and is approaching fast.

2. In Situ Transcriptomics to Map Cells, Molecules, and Genetic Variance Across Tumor Tissue Sections

Mats Nilsson[1]

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I will present the targeted in situ transcriptomic method *in situ* sequencing, and its application to spatial mapping of normal and disease tissue sections. The method involves panels of barcoded padlock probes that are hybridized, ligated, and then amplified by RCA to generate clonally amplified micron-sized DNA molecules that can be decoded by next-generation sequencing or sequential combinatorial hybridization reactions, allowing highly multiplex RNA expression and mutation analysis in situ. We generate and analyze such data by in-house chemistry and by using the Xenium instrument. We apply the method to study molecular, cellular and genetic heterogeneity in tumors, but also to create spatial atlases of normal organs.

Abstracts of invited lectures

1. 3D spheroids for genotoxicity assessment

Marc Audebert[1]

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Genotoxicity testing has traditionally relied on two-dimensional (2D) *in vitro* cell models, which are limited in their ability to replicate the complex metabolic interactions observed *in vivo* and are restricted to acute 24-hour treatment. In recent years, three-dimensional (3D) *in vitro* cell models have emerged as potentially more physiologically relevant systems. However, they have not allowed for high-throughput genotoxicity screening and have not been standardised. Within the framework of the European PARC project, we developed a human multicellular liver spheroid containing the HepaRG cells (hepatocytes and cholangiocytes), the hTERT-HSC cell line (Kupffer cells), and the THP-1 cells (immune cells) to create metabolically competent human liver spheroids. We were able to grow spheroids, perform acute or repeated chemical treatments, indirect antibody immunofluorescence staining and confocal imaging on the same plate, with four uniform spheroids per well. An image analysis workflow was then developed using 3D reconstructed images. We demonstrated the important metabolic capacity of this 3D model. Chemical genotoxicity modes of action evaluation with gH2AX and pH3 will be presented, and the difference between the 2D and 3D cell models will be highlighted. The difference between acute and repeated treatment will also be detailed. Other specific biomarkers related to inflammation and oxidative stress have also been implemented in this model. Using these spheroids, we were able to detect genotoxic potential for some chemicals not efficiently detected in 2D models. This human multicellular liver spheroid model may allow more efficient identification of chemicals with genotoxic potential in the future.

2. Advancing *In Vitro* Genotoxicity Testing with 3D Liver Models and Duplex Sequencing

Gillian E Conway[1]

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Accurate genotoxicity testing is critical for evaluating the risks posed by chemical exposures. Conventional assays—including the Ames test, mammalian cell mutation assays, and transgenic rodent models—are informative but limited by labour intensity and reliance on animal systems. In this study, we investigated the utility of error-corrected next-generation sequencing (ecNGS), specifically duplex sequencing (DS), in combination with an advanced three-dimensional (3D) liver model for *in vitro* mutation detection. A robust and adaptable 3D HepG2 spheroid system was established, demonstrating sustained viability and liver-specific functionality over a 14-day culture period. Repeated dosing with aristolochic acid over four days induced a dose-dependent increase in micronucleus formation, confirming the presence of fixed DNA damage. Application of DS, coupled with mutational signature analysis, revealed a treatment-specific T:A > A:T substitution pattern. These results validate the 3D HepG2 spheroid model as a physiologically relevant platform for assessing chemically induced DNA damage and highlight the capacity of DS to identify mutational signatures characteristic of mutagenic exposures. Together, this integrative strategy represents a cost-effective and efficient alternative to conventional genotoxicity assays, improving the precision of hazard evaluation and advancing mechanistic insights into chemical carcinogenesis.

3. RNA-seq–Validated Dynamic 3D Organ Models Mirror Human Tissues for Predictive Toxicology

Marta Sendra[1]

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Three-dimensional (3D) culture systems under static and especially under dynamic 3D models stimulation are redefining experimental fidelity by recapitulating tissue architecture, gradients, and cell–cell communication that are absent in 2D monolayers. Using transcriptome-wide RNA-seq, these models have been benchmarked against *in vivo* references to quantify their biological realism. Correlation analyses with the Human Protein Atlas revealed strong concordance between organ-specific signatures in dynamic 3D cultures and native tissues: liver constructs recovered xenobiotic metabolism pathways (e.g., CYP450 and UGT enzyme networks), neuronal models re-established synaptic transmission, axon guidance, and neurotrophic signalling, and lung equivalents reinstated epithelial barrier modules including tight-junction assembly, surfactant production, innate immune sentinels, and ciliated cell programs. Pathway enrichment and cell-type deconvolution further showed that perfusion enhances metabolic zonation, electrophysiological gene sets, and mucociliary differentiation, respectively, highlighting the added value of biomechanical cues. Functionally, when challenged with emerging contaminants such as BPA and BPs analogues, 3D models produced dose–response profiles and modes of action that more closely mirrored human tissue responses, including cell viability, oxidative stress and genotoxicity. Together, these data demonstrate that RNA-seq-driven validation against Human Protein Atlas references elevates 3D dynamic models from heuristic surrogates to mechanistically anchored proxies of human organs and tissues, enabling more predictive toxicology and risk assessment for contemporary environmental exposures.

4. Immune-modulating effects on tumor-draining lymph nodes following neoadjuvant chemoradiotherapy combined with immunotherapy in patients with T3-4N0-2 NSCLC

S. Koomen[1], E. Ulas[1], A. Vrijmoet[3], I. Houda[2], C. Dickhoff[4], I. Bahce[2], S. Senan[5], T. de Gruijl[6], M. Fransen[2], T. Radonic[3], F. Schneiders[5], **F. van Maldegem**[1]

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Tumor-draining lymph nodes are central hubs where the immune system encounters tumor antigens and mounts protective responses. Yet, little is known about how standard treatments for non-small cell lung cancer (NSCLC)—particularly neoadjuvant chemoradiotherapy—shape the immune environment of these nodes. As immunotherapy increasingly enters curative-intent regimens, understanding these interactions has become essential.

In our work, we investigated how combining immunotherapy with conventional neoadjuvant treatment influences the immunological landscape of tumor-draining lymph nodes in patients with resectable stage T3-4N0-2 NSCLC. Using a range of analytical approaches, including histological staining and spatial transcriptomics, we characterized the immune-modulating effects of chemoradiotherapy with and without checkpoint inhibitors.

We observed that the addition of immunotherapy strengthened type I immune responses and enhanced the presence of effector and regulatory T cells within irradiated nodes, regardless of the level of radiation exposure. Meanwhile, higher radiation doses were linked to structural remodelling processes such as fibrosis and tissue repair signatures, yet these changes did not appear to compromise immune activation. Importantly, the immunotherapy component helped balance the wound-healing dynamics induced by radiation, supporting sustained immune activity.

These findings highlight the capacity of immunotherapy to amplify anti-tumor immune responses in lymphoid tissues that are both targets of, and collateral participants in, multimodal cancer therapy. Our results provide insight into how future treatment strategies might best integrate immunotherapy with conventional modalities to optimize systemic anti-tumor immunity.

5. Stereotactic Radiosurgery Treatment using HyperArc Technology for Brain Tumors

Marija Skoblar Vidmar[1,2]

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Stereotactic radiosurgery (SRS) is a highly precise form of external beam radiotherapy that delivers a biologically effective high radiation dose to a well-defined and immobilized intracranial target volume, typically in a single session or in up to five fractions. The treatment is characterized by a steep dose gradient at the margin of the target, ensuring maximal sparing of surrounding healthy brain tissue, with the primary aim of achieving local tumor control or cure. Over the past five decades, SRS has become an essential modality for treating malignant and benign brain tumors, functional neurological disorders, vascular malformations, and neuralgias. Technological progress in radiotherapy, particularly image-guided radiation therapy (IGRT), has enabled further refinement of SRS. One of the most recent advancements is HyperArc, a cutting-edge software solution integrated into the Varian TrueBeam system. Since its implementation at the Institute of Oncology Ljubljana in June 2020, HyperArc technology has been used to treat over 1,000 patients, demonstrating its integration into routine clinical practice. It enables high-precision treatment of multiple lesions in the brain with a single isocenter – the number of targets that can be addressed with one isocenter seems to be unlimited. It is most useful in the local treatment of brain metastases, which are an increasingly frequent cause of morbidity and mortality in patients with malignant diseases. Local control of each irradiated lesion and possible late toxicity are increasingly clinically important, which may affect not only survival but also neurocognitive function and quality of life.

6. Current challenges in the treatment of glioblastoma: Clinical aspects and research advances

Andrej Porčnik[1], Metka Novak[2], Barbara Breznik[2], Borut Prestor[1]

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Glioblastoma (GBM) remains the most aggressive primary brain tumor, with limited long-term survival despite significant advances in surgery, radiotherapy, and systemic therapies. Surgical resection continues to be a cornerstone of treatment, with the extent of tumor removal strongly correlated with patient prognosis. However, the infiltrative nature of GBM and its frequent proximity to eloquent brain areas present substantial challenges to achieving radical tumor resection.

From a neurosurgical perspective, current challenges in GBM management will be addressed, with a focus on intraoperative decision-making and the application of advanced imaging techniques during surgery. Achieving maximal safe resection requires a careful balance between excising infiltrative tumor tissue and preserving critical neurological functions. In this context, advanced intraoperative imaging, functional mapping and intraoperative neuromonitoring have become indispensable tools for optimizing surgical outcomes.

We will also present clinical experience and outcomes from the Department of Neurosurgery at the University Medical Centre Ljubljana, highlighting surgical strategies and their impact on patient prognosis. Furthermore, we will underscore the value of ongoing collaboration with the National Institute of Biology, which fosters integration between clinical practice and basic science. Current joint research efforts focus on understanding the GBM tumor microenvironment, invasion mechanisms, and treatment resistance, with the goal of identifying novel therapeutic targets and biomarkers to support personalized treatment approaches.

7. Circular RNAs in Liver Cancer: From Functional Characterization to Biomarker Discovery

Hana Trček[1], Rok Razpotnik[1], Benjamin Bajželj[1], Marija Lazić[1], Martin Zaplotnik[2], Blaž Trotovšek[2], Mihajlo Đokić[2], Miha Petrič[2], Boštjan Plešnik[2], Irena Plahuta[3], Arpad Ivanecz[3], Linda Cellner[2], Alojz Šmid[2], Rado Janša[2], Robert Vidmar[4], Marko Fonović[4], Uršula Prosenc Zmrzljak[5], Damjana Rozman[1], **Tadeja Režen**[1]

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Liver cancer is a major global health concern, ranking as the sixth most diagnosed cancer and the third leading cause of cancer-related deaths. Circular RNAs (circRNAs), a class of covalently closed RNAs, have emerged as promising biomarkers and RNA-based therapeutic in oncology. Through analysis of public microarray datasets, we identified hsa_circ_0062682 as differentially expressed in hepatocellular carcinoma (HCC) tissue. Functional characterization confirmed its oncogenic role in HCC cell lines. Transcriptomic analyses revealed widespread gene expression changes upon modulation of hsa_circ_0062682, and proteomic profiling identified its interaction with YBX1, a known oncogene. Interestingly, hsa_circ_0062682 was significantly downregulated in both tumour tissue and plasma in the Slovenian HCC cohort, characterized by non-viral, metabolic, and alcohol-related aetiology. To validate and expand these findings, we performed long- and short-read sequencing of circRNAs from paired tumour and adjacent non-tumor tissues. A meta-analysis of published circRNA datasets revealed limited overlap in differentially expressed circRNAs, highlighting platform-specific detection biases. Finally, we correlated tissue and plasma circRNA profiles to assess the biomarker potential of circRNA for liquid biopsy applications. In conclusion, before circRNAs can be implemented in clinical practice, a deeper understanding of inter-patient variability and rigorous validation and standardization of analytical procedures are essential.

This work was funded by the Slovenian Research and Innovation Agency (ARIS) grants J3-4513, P1-0390, IP-0022 (CFGBC), ESFRI-ELIXIR, the Ph.D. grants, and HPC RIVR consortium and EuroHPC JU by providing computing resources of the HPC system Vega at the Institute of Information Science.

Sponsor invited talk

8. Mapping the Tumor Microenvironment in Lung Cancer With High-Parameter Imaging Mass Cytometry

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The tumor microenvironment (TME) plays a critical role in lung cancer progression, therapeutic resistance, and response to immunotherapy. High-parameter Imaging Mass Cytometry (IMC) enables simultaneous spatial analysis of dozens of protein markers at single-cell resolution, providing an in-depth view of the cellular composition, organization, and functional states within tumor tissues. By preserving spatial context, IMC allows for the identification of distinct cellular neighbourhoods, immune infiltration patterns, and spatially regulated expression of checkpoint molecules. Combined with suspension mass cytometry, which enables high-dimensional phenotyping of dissociated cells, this technology offers a comprehensive approach to characterizing both the composition and architecture of the lung TME. Applications of IMC in lung cancer have revealed critical insights into immune-tumor interactions and stromal heterogeneity, supporting its growing role in translational research and biomarker discovery.

Abstracts of short lectures

1. Lung co-culture at the pseudo-air-liquid interface: A model for assessing genotoxic and inflammatory effects of airborne pollutants

Matjaž Novak[1], Martina Štampar[1], Alja Štern[1], Michael J. Burgum[2], Gillian E. Conway[2], Goran Gajski[3], Shareen H. Doak[2], Bojana Žegura[1]

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Indoor air pollution is an increasing public health concern, with airborne pollutants such as polycyclic aromatic hydrocarbons (PAHs) implicated in respiratory diseases and carcinogenesis. To better model lung exposure and assess the toxicological effects, we developed a co-culture model of a lung adenocarcinoma-derived A549 alveolar epithelial cells and differentiated THP-1 macrophage-like immune cells at the pseudo-air-liquid interface (pseudo-ALI). This setup closely mimics the human lung microenvironment by enabling physiologically relevant surfactant production and robust immune responsiveness, essential for accurately modeling airway exposure to inhaled pollutants. We investigated the genotoxicity and inflammatory responses to benzo[g,h,i]perylene (BGP) and benzo[b]fluoranthene (BBF), two PAHs commonly found in polluted air, using a co-culture model under pseudo-ALI conditions. After 24 hours of exposure to non-cytotoxic concentrations (BGP ≤18.1 µM; BBF ≤39.6 µM), neither compound induced DNA double-strand breaks (γH2AX) and chromosomal damage (cytokinesis-block micronucleus assay). However, both compounds elicited notable inflammatory responses. BGP significantly elevated IL-8 secretion and upregulated TNF-α, IL-6, and IL-1β expression, as confirmed by ELISA and flow cytometry. BBF similarly increased the percentage of cells expressing TNF-α and IL-6, along with IFN-γ. Targeted gene expression analysis revealed upregulation of multiple inflammation-associated genes (*IL-8*, *IL-6*, *IL-1β*) following exposure to both PAHs. Our findings demonstrate that co-culture at the pseudo-ALI exposure effectively captures early inflammatory responses to airborne toxicants, offering a physiologically relevant platform for evaluating inhalation hazards and their potential long-term health impacts.

Supported by HEU CutCancer (101079113), HEU EDIAQI (101057497), ARIS (P1-0245).

2. *In vitro* 3D hepatic co-culture model for assessing adverse chemical effects

Tim Ravnjak[1,3]**,** Gillian Conway[2], Michael J. Burgum[2], Shareen H. Doak[2], Bojana Žegura[1,3]

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In vitro hepatic cell models are considered one of the cornerstones of genetic toxicology. Traditionally, experiments are conducted on 2D monolayer cultures; however, these models poorly mimic the complexity of liver tissue. To address these limitations, an array of new approaches to establish *in vitro* hepatic 3D cell models have been developed recently with enhanced liver-specific functions, including the expression of phase II. xenobiotic metabolic enzymes. However, the majority of currently used hepatic 3D cell models are based on a single cell type, despite the liver being a multicellular organ composed of diverse cell type populations. In response to this limitation, we established a 3D hepatic co-culture model comprising HepG2 hepatocellular carcinoma cell line and liver-specific macrophage Kupffer cells (KC). The three-day-old HepG2/KC co-culture model was exposed to the carcinogens griseofulvin (GF; 2-200 µM) and cadmium chloride ($CdCl_2$; 0.25-25 µM) for 24h. Cell viability was assessed with the LDH assay, liver functionality via albumin secretion, inflammatory response by ELISA (IL-6, IL-8, TNFα) and genotoxicity with the comet assay. Both chemicals decreased viability at the highest tested concentrations, whereas there was no observed effect on albumin expression. Of the inflammatory markers assessed, both chemicals stimulated the release of IL-8, with $CdCl_2$ triggering a dose-dependent response. In contrast, neither IL-6 nor TNFα were detected following exposure to either chemical. Notably, $CdCl_2$ significantly increased DNA damage, as indicated by elevated tail intensity in the comet assay. To gain deeper insight into molecular mechanisms underlying these effects, further transcriptomic analysis of gene expression is underway.

Funded by HEU project CutCancer (101079113) and ARIS [P1-0245]

3. Pushing Boundaries: Scientific Milestones of CutCancer

Barbara Breznik[1], Metka Novak[1], Alja Štern[1], Febe van Maldegem[2], Mats Nilsson[3], Shareen H. Doak[4], **Bojana Žegura**[1]

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At the National Institute of Biology (NIB), as part of the HEU Twinning project *CutCancer*, we have significantly advanced our expertise and capacity for innovative solutions in carcinogenesis and cancer research through the integration of cutting-edge 3D cell models and methodologies. Building on these methodological advancements, the project established novel 3D liver models, developed both as monocultures and co-cultures, and lung co-culture models, capturing the intricate cellular complexity and intercellular interactions characteristic of human organs. These physiologically relevant platforms enabled the detailed assessment of DNA damage and genomic instability, critical hallmarks of cancer, in response to exogenous factors such as chemicals (1) and nanoparticles (2). Coupled with automated high-throughput analyses using Metafer MetaSystems (3), these models allow rapid, reliable, and quantitative evaluation of micronuclei, DNA strand breaks, and other genotoxicity endpoints. Applied to chemical and nanoparticle risk assessment, these novel *in vitro* systems provide mechanism-based, high-fidelity platforms that enhance predictive preclinical studies while minimizing reliance on animal testing.

Further advancing the project goals, organoid models were developed to replicate the complex tumor microenvironment observed in cancer patients (4,5). By leveraging novel spatial biology techniques, we can visualize tumor cellular composition and map cellular and molecular interactions, enabling us to monitor therapeutic responses within a spatial context. In situ sequencing (ISS) and Imaging Mass Cytometry (IMC), used independently and in combination, have been instrumental in this effort. Applied to tumor organoids that mimic complex TMEs, these technologies offer powerful platforms for identifying novel biomarkers and developing personalized treatment strategies. Using the Slovenian translational platform *GlioBank* alongside publicly available datasets, we have identified promising biomarkers for aggressive brain tumors—specifically MAGE type I and DAB2. These markers are associated with distinct patient subgroups and

tumor progression, and their targeting may represent a viable therapeutic strategy for the most malignant forms of childhood and adult brain cancers (4,6).

To foster innovation, creativity, and strengthen international collaborations, NIB has focused on advancing genetic toxicology and translational preclinical 3D cancer research. These efforts have elevated NIB to a level of scientific excellence, enhancing its international recognition and competitiveness. As a result, NIB has established itself through leadership roles in multiple European and international project consortia. Several joint project proposals have been successfully awarded through competitive EU and ARIS national calls, including the EU Horizon Cancer Mission, HEU-RIA, MSCA-PF, and M-ERA.NET international projects, as well as an ARIS Strategic project, and an ERC perspective projects supported by ARIS.

The integration of novel methodologies at NIB has catalyzed the development of state-of-the-art knowledge and capabilities in carcinogenesis and cancer research. These advances have enabled mechanistic insights through 3D cell models for genotoxicity assessment and have initiated new preclinical studies focused on personalized medicine, aimed at discovering and validating novel cancer biomarkers and therapeutic strategies.

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4. Enabling Innovation and Excellence: Research Support in Action

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The CutCancer project, funded under the Horizon Europe program, exemplifies a strategic and systemic approach to advancing research excellence through robust support infrastructures. Strategic research support plays a pivotal role in advancing scientific excellence, particularly in complex fields such as 3D *in vitro* cancer research. We established a multi-dimensional approach to enabling innovation through structured data governance, targeted capacity building, and rigorous ethical oversight.

A robust data management framework is being implemented to ensure the integrity, security, and accessibility of research data. Emphasising FAIR principles - Findability, Accessibility, Interoperability, and Reusability - the framework supports open science practices and compliance with legal and ethical standards, including GDPR and intellectual property rights. Defined roles and responsibilities across data stewards, managers, and users foster accountability and transparency throughout the data lifecycle.

Capacity building efforts extended beyond researchers to include corporate service staff, whose expertise in administration, finance, legal, and data protection proved essential to the project's success. Staff exchanges and expert visits enabled cross-institutional learning, fostering a shared understanding of operational challenges and solutions. These exchanges strengthened institutional capabilities, enhanced support structures, and cultivated a collaborative culture that benefits both administrative and scientific teams.

The CutCancer project adheres to the highest standards of research integrity, in line with the European Code of Conduct for Research Integrity. This includes strict avoidance of fabrication, falsification, plagiarism, and other forms of misconduct. Ethical compliance is further ensured through detailed guidelines covering research reproducibility, use of cell lines and organoids, handling of hazardous substances, patient consent, personal data protection, and safety during international activities. All research and project activities were conducted ethically and in full compliance with national and international regulations.

To maximize the impact of the CutCancer project, a Dissemination, Exploitation, and Communication plan was developed. Relevant stakeholders, including the scientific community, citizens, cancer patients and their families, clinicians, and policymakers, were engaged through various activities aimed at enhancing

CutCancer's contributions to the development of new ideas and breakthroughs in cancer research, while also increasing interactions with key stakeholders.

This inclusive approach helped secure new European and national research projects by strengthening institutional capacity and knowledge transfer, showing how strategic investment drives scientific excellence and sustainable innovation in cancer research.

Supported by the HEU project CutCancer [101079113] and the Slovenian Research and Innovation Agency [P1-0245].

5. Advanced Spatial Technologies for Visualization of Immunosuppressive Glioblastoma Microenvironment

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Glioblastoma (GB) is the most common primary brain tumor with poor overall survival. One of the main reasons for ineffectiveness of the treatment is highly immunosuppressive GB microenvironment and therapy-resistant cancer cells residing in specific cancer cell niches. Cancer cells interact with non-cancerous cells in tumor microenvironment e.g. immune cells, vascular cells, fibroblasts and thus influence response to therapy. To improve the patients' response to treatment, we need to understand and possibly target interactions within GB microenvironment.

To investigate the composition of the glioblastoma (GB) microenvironment and detect cancer cell niches, we have employed techniques such as immunofluorescence (IF) and bulk transcriptomics. Each method, however, has its limitations. IF enables us to identify and localize specific cell types within the GB microenvironment, but the number of detectable markers is constrained by the limited availability of fluorophores. As a result, we can only analyze a small subset of cell markers. In contrast, bulk transcriptomics provides a comprehensive overview of the cellular composition, yet it lacks spatial resolution, making it impossible to determine the precise spatial localization of individual cell populations within the sample. To overcome these limitations, we introduced into our lab two novel spatial technologies, in situ sequencing (ISS) and Imaging Mass Cytometry (IMC). With ISS and IMC, we can visualize a much higher number of selected targets within a single tumor sample that can give us a more complete insight into GB microenvironment.

Neither of these methods is currently in use in Slovenia, which is why we seized the opportunity – through the CutCancer project – to visit SciLifeLab in Stockholm and

University Medical Center in Amsterdam to learn ISS and IMC, respectively. During intensive hands-on training, we became familiar with every step of wet lab and analysis pipeline and refined our skills to the point where we can now apply these methods independently and with confidence. ISS has been implemented in our lab to address our research questions, while IMC will still require collaboration with our international partners.

During hands-on trainings, we have applied technologies to patient-derived GB tissue sections and organoids. First, we created and optimized IMC and ISS target panels, which we then used to look into changes that occur within GB microenvironment upon treatment. As a part of CutCancer project we have also been working on optimizing protocols for ISS and IMC and trying to combine both technologies on the same slide.

ISS and IMC are advanced spatial technologies which give us insight into composition and interactions within GB microenvironment with high resolution. Combining both technologies is necessary for a better understanding of mechanisms underlying therapy resistance and immunosuppression in glioblastoma patients, as well as for finding new potential biomarkers and therapeutic targets.

Abstracts of posters

1. Hepatic 3D cell model for assessment of genotoxic effects induced by benzo[a]pyrene (B[a]P) and dibenz[a,h]anthracene (DB[a,h]A)

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants generated during the incomplete combustion of organic materials, vehicle emissions, industrial processes, and indoor activities such as cooking and smoking. Among them, benzo[a]pyrene (B[a]P) and dibenzo[a,h]anthracene (DB[a,h]A) are classified as highly potent carcinogens. While the genotoxicity of B[a]P is well documented, limited information is available on the genotoxic mechanisms of DB[a,h]A. To address this knowledge gap, a physiologically more relevant 3D HepG2 (human hepatocellular carcinoma) spheroid model was employed to evaluate and compare the genotoxic effects of B[a]P and DB[a,h]A. Cytotoxicity (ATP assay) and genotoxicity (comet assay, flow cytometry for γ-H2AX, P21, and pH3) of both compounds were evaluated after 24 (short-term) and 96 hours (long-term) exposures, together with targeted gene expression analysis to assess their mechanisms of action. At non-cytotoxic concentrations (5 µM and 0.25 µM for 24 and 96 hours, respectively), dose-dependent DNA damage was detected at both exposure time points for both compounds. Gene expression analysis revealed upregulation of key genes involved in DNA damage response, oxidative stress, and xenobiotic metabolism (encoding phase I and II enzymes). Flow cytometric analysis confirmed increased levels of DNA damage-associated markers (γ-H2AX). Collectively, our results demonstrate that DB[a,h]A exhibits genotoxic potency comparable to B[a]P, with consistent effects across multiple endpoints. These findings underscore the relevance of 3D hepatic models in toxicological assessment and emphasise the need for further mechanistic studies to elucidate the toxicological profile of DB[a,h]A and support accurate risk assessment and public health protection.

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2. Establishment of Physiologically Relevant *In Vitro* Models of Natural Killer Cell–Glioblastoma Interactions

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Glioblastoma (GB) is an aggressive type of brain cancer with poor patient prognosis and limited treatment options. Natural killer (NK) cell-based immunotherapy is a promising approach for GB treatment. As executioners of innate immunity, NK cells can eliminate a spectrum of target cells without prior sensitization. Importantly, it has been demonstrated that they can also eliminate glioblastoma stem cells (GSCs), which are intrinsically resistant to standard therapeutic approaches and drive GB recurrence. Nevertheless, NK cell function may be hampered in the immunosuppressive GB tumor microenvironment. To better understand the interactions between GB and NK cells, we set up three distinct physiologically relevant *in vitro* model systems. Firstly, we established spheroids from either GSCs or differentiated GB cells in the Celvivo Clinostar system and subsequently co-cultured them with NK-92 cells. NK-92 cells more efficiently infiltrated spheroids of differentiated GB cells, but their cytotoxicity was higher against GSC spheroids. In concordance with substantial NK-92 infiltration, NIB140 spheroids secreted higher levels of immunomodulating and NK cell-attracting cytokines. Secondly, NK-92 infiltration towards GB was studied in a dynamic microfluidic platform mimicking the blood flow and influx of immune cells into the tumors, which confirmed higher NK-92 cell attraction of differentiated GB spheroids compared to GSC spheroids. Thirdly, the ability of NK-92 cells to detect and eliminate GB cells was investigated in advanced co-cultures of GB spheroids and brain organoids. Altogether, our *in vitro* model systems present a groundwork for further studies of the complex crosstalk between GB and NK cells.

3. Impact of APOBEC3A and APOBEC3B Silencing on p53 Expression in Human Foreskin Keratinocytes

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APOBEC3 proteins are cytidine deaminases that belong to the AID/APOBEC (Activation-induced deaminase/Apolipoprotein B mRNA editing catalytic polypeptide-like) family. They catalyze the deamination of cytosine to uracil in single-stranded DNA or RNA and act as general antiviral factors in innate immunity, restricting the replication of retrotransposons, RNA viruses and DNA viruses (e.g. human papillomavirus). Cytosine deamination induces hypermutation of viral genome, leading to reduced viral fitness and inactivation of the virus. However, dysregulation of this mechanism can trigger hypermutation of host genome and contribute to cancer development. APOBEC mutation signatures are the second most common in human cancers, with APOBEC3A and APOBEC3B being the main drivers of these mutations. APOBEC3 proteins also promote cancer progression, evolution and the development of cancer therapy resistance.

The aim of our study was to explore the role of APOBEC3 proteins in carcinogenesis and the underlying mechanisms. To explain their effect on tumorigenesis in human foreskin keratinocytes (HFKs), we investigated the impact of APOBEC3 silencing on the tumor suppressor p53. Expression of p53 was assessed in wild-type and HPV-infected HFK cell lines by western blot, qPCR and immunofluorescence. We observed an upregulation of p53 protein levels following APOBEC3A and APOBEC3B silencing with siRNA. Together with previous studies, these results indicate that APOBEC3A and APOBEC3B expression is inversely related to p53 status, suggesting that loss of p53 leads to increased expression and activity of both deaminases associated with higher mutagenic activity and cancer development.

4. Survivors Become Invaders: Electroporation-Induced Changes in Glioblastoma Cell Behavior

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Introduction**:** Electroporation is a promising approach in glioblastoma (GB) therapy, enabling drug delivery and nonthermal ablation. However, little is known about how sublethal electric fields affect the behaviour of surviving tumor cells. This study investigates short-term effects of electroporation on the invasiveness of patient-derived GB cells.

Methods: Five GB cell lines were screened for baseline invasiveness; two highly invasive lines (NIB140 CORE, NIB216 CORE) were selected for further analysis. Cells were exposed to high-frequency irreversible electroporation pulses at varying electric field strengths. Membrane permeabilization, viability (PI/MTS), and transwell invasion were assessed 24 hours post-treatment. RNA sequencing was performed to identify gene expression changes related to altered invasiveness.

Results: Both cell lines showed increased membrane permeabilization following electroporation, with NIB216 CORE exhibiting greater sensitivity and reduced viability at higher field strengths. At 1.0 kV/cm—a field strength commonly used in electrochemotherapy (ECT)—both lines displayed increased invasiveness (up to 4.23-fold in NIB140 CORE), without corresponding changes in proliferation (Ki-67). RNAseq analysis revealed differentially expressed genes between treated and control samples, including several involved in ion transport and cytoskeletal regulation.

Conclusion: Sublethal electroporation may promote invasive behaviour in GB cells. To validate and extend these findings, future studies should employ advanced 3D models (e.g., spheroids, organoids), include stem-like and region-specific tumor cells, and evaluate long-term outcomes and treatment combinations. Deeper mechanistic insight will support the development of safer and more effective electroporation-based therapies for GB.

5. Unveiling the Potential of Electroporation-based Glioblastoma Treatment Using Patient-derived Biomimetic Models

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Glioblastoma is the most aggressive type of brain tumors, with current standard treatments achieving a median survival of ~15 months. Electroporation has emerged as a promising treatment modality that uses short, high-intensity electric pulses to either transiently increase cell membrane permeability for enhanced drug delivery (electrochemotherapy) or achieve non-thermal tumor ablation (irreversible electroporation). Encouraging results have been demonstrated in animal studies. However, neither canine nor rodent gliomas fully replicate the complexity of human GB. This project aims to systematically investigate electroporation effects using state-of-the-art biomimetic models of increasing complexity. First, we will use patient-derived primary GB cells, which, unlike established cell lines, much more faithfully reproduce key aspects of tumor biology. Second, we will employ 3D multicellular spheroids that better represent the microscopic electric field distribution in tumors and hindered diffusion of therapeutic agents. Third, we will pioneer the use of patient-derived GB organoids in electroporation research. We will assess treatment effects on tumor cell viability, proliferation and invasion, while carefully evaluating responses of therapy-resistant GB stem cells, immune cells, and surrounding healthy tissue.

The project addresses fundamental questions that cannot be systematically investigated *in vivo* due to ethical constraints and experimental complexity. Our methodological approach enables investigation of how surviving tumor cells respond to reversible electroporation, how cellular heterogeneity influences treatment efficacy, and how the immune system responds to the treatment. The insights gained will be essential for optimizing treatment protocols and identifying the most promising therapeutic combinations for clinical translation.

6. Cytotoxic activity of imidazolium oximes in prostate cancer cells (PC-3)

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Prostate cancer ranks among the most prevalent cancers and remains challenging to treat with existing therapeutic agents. Consequently, the search for novel compounds with improved antitumor potential is ongoing. Imidazolium oximes represent a promising class of such compounds, as their heteroaromatic structure confers a wide spectrum of pharmacological activities via diverse cellular targets and mechanisms. Given the therapeutic potential of oximes in inhibiting tumor progression, this study investigated the effects of newly synthesized hydroxyimino-methyl imidazolium bromides (IV, VI, VII, and X) on PC-3 prostate cancer cells. Cells were treated with oximes at concentrations ranging from 6.25 to 800 μmol dm^{-3} for 1 h, 4 h, and 24 h. Cytotoxicity was assessed by evaluating mitochondrial succinate dehydrogenase activity in metabolically active cells. In addition, lactate dehydrogenase (LDH) release was measured to determine membrane integrity, and flow cytometry was used to assess the potential induction of apoptosis. The results demonstrated that all tested hydroxyimino-methyl imidazolium bromides significantly reduced PC-3 cell viability in a concentration- and time-dependent manner. Among them, oximes VII and X—bearing an aromatic side chain—exhibited the strongest inhibitory effects and caused substantial LDH release, pointing to necrotic cell death. However, no evidence of apoptosis was observed. These findings suggest that structural modifications will be necessary for these oximes to be considered viable candidates for further development as antitumor agents.

Keywords: prostate cancer, PC-3 cells, imidazolium oximes, cytotoxicity, necrosis, apoptosis, antitumor agents

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7. Impact of Tumour Cell and Cancer-Associated Fibroblast Crosstalk on the Expression and Activity of Cysteine Cathepsins in the Tumor Microenvironment

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The tumour microenvironment (TME) comprises diverse cell types that interact and influence cancer progression. Among the key enzymes involved are cysteine peptidases, which contribute to tumour development and metastasis. Notably, the dynamic interplay between cancer cells and cancer-associated fibroblasts (CAFs) within the TME significantly impacts tumour growth, metastasis, and therapy resistance. This study aimed to investigate whether co-culturing tumour cells with CAFs modulates the expression and activity of specific cysteine peptidases, including cathepsins B, X, V, and L. To assess if cysteine peptidases secreted by one cell type influence expression in another, breast cancer cell lines were cultured for three days, and their conditioned media were applied to CAF cultures, and vice versa. Cell lysates were collected at 0, 24, and 72 hours for analysis. Protein expression and enzymatic activity of the target cathepsins were measured using western blotting and enzyme activity assays.

Our findings demonstrate that co-culturing cells or exposing one cell type to conditioned media from another leads to altered protein levels and activity of cathepsins. These results indicate that cysteine cathepsins play a crucial role in mediating communication between different cell populations within the TME. In summary, this study reveals that crosstalk between tumour cells and CAFs regulates cysteine peptidase expression and activity. Understanding this interaction is essential and should be factored into the development of more effective anti-tumour therapies.

8. Impact of tumor microenvironment on mitochondrial ultrastructure and viability of glioblastoma stem and differentiated cells

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Glioblastoma stem cells (GSCs) exhibit remarkable plasticity, enabling rapid adaptation to therapeutic pressures and contributing to poor prognosis in glioblastoma (GB). This plasticity involves dynamic metabolic and phenotypic shifts influenced by the tumor microenvironment (TME), including the perivascular niche where endothelial cells reside. The interaction with signalling molecules of endothelial cells through conditioned media modulates GSCs mitochondrial ultrastructure and function, supporting their survival and resistance to chemotherapy.

In our study, we investigated the impact of conditioned media of endothelial cells, on the metabolic viability and mitochondrial architecture of two GSC and two differentiated GB cell lines, following treatment with temozolomide (TMZ). Transmission electron microscopy demonstrated that mitochondria in GSCs predominantly retained normal, electron-dense morphology with narrow cristae, indicative of active oxidative phosphorylation, whereas differentiated GB cells displayed variable mitochondrial defects and greater reliance on glycolysis. Exposure to growth and conditioned media of endothelial cells promoted a shift of mitochondrial structure towards normal in both cell types, highlighting the microenvironment's role in modulating metabolic plasticity. Importantly, co-treatment with TMZ and conditioned media selectively reduced viability in differentiated GB cells without compromising GSCs viability or mitochondrial integrity. These findings reveal the adaptive metabolic plasticity of GSCs and suggest that the perivascular niche may provide protective signals that specifically benefit GSCs during chemotherapeutic stress.

9. Automated Quantitative Fluorescence Microscopy and Image Analysis for *In Vitro* Toxicological Evaluation

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With the increasing number of chemicals requiring safety evaluation, there is a need for reliable, physiologically relevant *in vitro* models for genotoxicity testing. Three-dimensional (3D) hepatic spheroids offer improved structural and metabolic relevance compared to traditional 2D cultures and present a promising alternative to animal testing. Automated quantitative fluorescence microscopy, combined with advanced image analysis, enables rapid, cost-effective, and high-content toxicological screening, particularly for multiplexed and time-dependent studies. This study aimed to optimize an automated fluorescence imaging and analysis workflow using the Cytation 5 Microplate Reader (Agilent BioTek), with validation on the EVOS M7000 Imaging System. *In vitro* 2D and 3D HepG2 liver models were used for (geno)toxicity screening through automated fluorescence microscopy and quantitative image analysis. 2D cultures were seeded in 96-well plates and cultured for 24 hours. 3D spheroids were formed in AggreWell plates and maintained for 21 days in ClinoStar bioreactors (CelVivo) under dynamic conditions. Spheroids were embedded in glycerol and transferred into black 96-well plates or sectioned into 5 mm paraffin slices. Both models were exposed to two airborne polycyclic aromatic hydrocarbons (PAHs)—benzo[b]fluoranthene (BBF) and benzo[g,h,i]perylene (BGP)—individually and in combination. Method optimization employed reference genotoxicants: benzo[a]pyrene, etoposide, and tert-butyl hydroperoxide. Post-treatment, cells were fixed and immunostained for key biomarkers of proliferation (Ki67), DNA damage (γH2AX, p-H3, PARP1), and oxidative stress (HMOX1, SRXN1). Imaging data were analyzed using Gen5, Celleste, and ImageJ with semi-automated segmentation and quantification. The study revealed clear differences between 2D and 3D responses across endpoints, consistent with existing toxicological data. Findings support automated fluorescence microscopy as a robust approach for high-throughput toxicity screening. Future work will expand biomarker panels and advance automated workflows for confocal imaging.

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10. Influence of siRNA Targeting B7-H3 on Glioblastoma-Microglia Spheroids and *In Silico* Optimization of Nanobody Affinity

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Glioblastoma is the most aggressive and lethal malignancy of the central nervous system. With current standard of care, patient survival somehow remains stable over the past two decades. Immunotherapeutic approaches, including monoclonal antibodies, CAR-T cells, antibody-drug conjugates, and nanobodies, are becoming more attractive and show encouraging results. Due to the small size and better tissue penetration, nanobodies are leading the next generation of therapeutic antibodies, with four already approved for clinical use since 2018.

Here we focus on the immune checkpoint B7-H3, which is upregulated in glioblastoma and is associated with poor prognosis and metastatic behavior. Using five primary glioblastoma cell lines (NIB140, NIB142, NIB169, NIB261R, and NIB237), we optimized co-culture conditions for glioblastoma-microglia cells and developed two spheroid models: 50/50 (equal ratio) and 60/40 (glioblastoma-enriched). Spheroid morphology (cell size, circularity, surface area) was analyzed on Agilent Biotek Lionheart FX. We used siRNA silencing to assess the functional relevance of B7-H3. B7-H3 silencing significantly reduces cell size and surface area of NIB142 spheroids (both 50/50 and 60/40 models) after 3 days ($p<0.05$). Similar effects were observed for NIB140 and NIB169 spheroids (60/40, $p<0.05$) after 6 days. All spheroid types showed consistent decrease in spheroid size and surface area ($p<0.0001$) with no change in circularity. Moreover, we optimized *in silico* the binding affinity of anti-B7-H3 nanobody NB63. However, because of high variability, we could not confirm variants with increased binding affinity. To conclude, we successfully established glioblastoma–microglia co-culture spheroids and demonstrated that B7-H3 silencing significantly affects spheroid morphology.

11. Cross-Species Validation of FREM2's Role in Glioma Progression: From Human Glioblastoma to a *Drosophila* Model

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Glioblastoma is the most aggressive primary brain tumor. Its hallmarks include high genetic heterogeneity, an immunosuppressive microenvironment, and the presence of treatment-resistant glioblastoma stem-like cells (GSCs). In our previous studies (PMIDs: 29734672, 31357584, 34439271 and 39982223), we identified and validated *FREM2* as a GSC-specific gene and hypothesized its role in tumor progression and therapy resistance.

Therefore, in this study using siRNA silencing, we examined the functional role of FREM2 in both stem-like (NCH644, NCH421K) and differentiated (U87MG, U251MG) glioblastoma cell lines. With transcriptomic analysis of silenced and control cells, we revealed notable changes in pathways linked to apoptosis, catabolic activity, and vesicle regulation. Among the differentially expressed genes we detected *CCND3*, *TMEM198*, *SEZ6L2*, *API5*, and *SAMD8*, some of which are already linked to cancer progression. *FREM2* silencing made glioblastoma cells more sensitive to temozolomide by increasing apoptosis. *API5* and *SAMD8* emerged as potential effectors of FREM2-mediated apoptotic resistance. Despite its location to the basal membrane, migration and invasion assays and analysis of cytoskeletal organization did not show differences between silenced and control cells. We also used *in vivo Drosophila* glioma model to confirm our *in vitro* findings. *In vivo* both up- and down-regulation of *Perdido* (*Drosophila* homologue of *FREM2*) affected glioma cell proliferation. Based on the results of our comprehensive functional study, we conclude that *FREM2* role in glioblastoma is likely related to apoptosis and survival mechanisms. FREM2 is a promising therapeutic target relevant for overcoming GSC-mediated resistance.

12. Ephrin B3: A Novel Therapeutic Target and Radiosensitizer in Glioblastoma

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Glioblastoma (GB) is the most common primary brain tumour with poor overall survival. To improve patients' response to treatment, signalling pathways linked with therapy resistance could be targeted. One of such is ephrin pathway, including Ephrin B3 and phosphorylated EphA2 S897, which are upregulated in GB and linked with tumour growth and invasiveness. The aim of the study was to explore the role of Ephrin B3 and EphA2 S897 signalling in GB radioresistance.

Patient-derived GB cell lines were established from fresh tumour tissue biopsies. Clonogenic potential and cell proliferation were studied after a single dose RT, fractionated RT, Ephrin B3 silencing, as well as after combination treatment (RT and Ephrin B3 siRNA). Differences in Ephrin B3 and EphA2 S897 expression and localisation were analysed by western blot and immunofluorescence.

Our results showed that RT decreased clonogenic potential of GB cells in dose-dependent manner, leading to reduced number and size of colonies. Ephrin B3 silencing led to decreased cell proliferation and as well as colony-forming capacity. Additionally, we observed that in response to treatment, cells formed more protrusions, in which Ephrin B3 and EphA2 S897 were present at high levels.

To reveal if Ephrin B3 silencing sensitizes GB cells to RT, we lastly combined both treatments. Clonogenic assay showed that Ephrin B3 silencing or 2 Gy RT itself reduced plating efficiency of GB cells; however, plating efficiency decreased for another 50 % when cells were exposed to both treatments.

Our results suggest that Ephrin B3 diminishes the responsiveness of GB cells to radiation therapy, suggesting that targeting Ephrin B3 could enhance radiosensitivity in GB.

13. Utilizing a Dynamic Microfluidic System for Exploring Glioblastoma and Natural Killer Cell Interactions

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Glioblastoma (GBM), a highly aggressive grade IV glioma, remains incurable despite aggressive conventional treatments. Heterogeneity and therapeutic resistance hinder efficient treatment; therapeutic resistance is primarily due to the complex tumor microenvironment (TME). There is little known about the immune TME and a lack of tumor models, which would mimic GBM-immune cell interactions and its dynamic components. Therefore, we set up an immune-competent glioblastoma *ex vivo* model to investigate immune cell infiltration into tumors and to examine the interactions between GBM and immune cells within the tumor microenvironment.

We set up a microfluidic platform (MIVO® platform, React4life) to better understand the role of GBM-immune cell interactions. Tumor cell spheroids were established from patient-derived GBM cells and GBM stem cells, embedded in Matrigel® and cultured in the tumor chamber above a microcirculation of natural killer (NK) cells, mimicking the influx of immune cells into the tumor. Cells were co-cultured for 24 hours at different effector-to-target ratios. Fluorescence microscopy and flow cytometry were used to determine the infiltration of labelled NK cells into the spheroids and the viability of GBM and NK cells.

We studied the infiltration of NK-92 cells into the GBM spheroids and how it affected the viability. To ensure viability of NK cells, flow rate in the circulation was optimized. Besides NK cells, GBM cells also remained viable during the experiment. We observed infiltration of NK cells into the tumor chamber after 24h, however, increasing the effector-to-target ratio did not affect the infiltration of NK cells into the chamber. On the other hand, increasing the serum in the tumor chamber did act as a chemoattractant and increased the infiltration of NK cells into the tumor chamber. Using fluorescence microscopy, we observed infiltration of labelled NK cells into the chamber and spheroids. Matrigel was easily removed using cold PBS and after spheroid dissociation, cells in the tumor chamber were further analyzed using flow-cytometry.

We established a microfluidic platform that mimics GBM-immune cell dynamic interactions in humans which could provide more insight to the immune component. In the future, we could extend the incubation time and use patient-derived immune cells and paired patient-derived organoids. The dynamic component of such *ex vivo* brain tumor model better replicates the *in vivo* conditions and can be used to further explore the biology of GMB and to test

combinational anti-cancer approaches, including targeted therapy and immunotherapy.

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14. Prostaglandin EP4 Receptor Activation Alters CLL Microenvironment and Potentiates Effects of Ibrutinib and Venetoclax

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Chronic lymphocytic leukaemia (CLL) is a B cell malignancy highly dependent on B cell receptor signalling for survival. Our findings suggest that targeting the prostaglandin EP4 receptor may offer a novel therapeutic approach. In this study, we evaluated the selective EP4 receptor agonist PgE1-OH for its anti-leukemic potential in CLL.

Prostaglandin EP4 receptor agonist PgE1-OH exhibited concentration- and time-dependent cytotoxicity in primary B cells obtained after informed consent from 155 CLL patients (Study Nr. 93/12/10). EC50 values were significantly lower in malignant B cells (13.56 µM) compared to lymphoblastoid cell lines (55.43 µM) and peripheral blood mononuclear cells (46.36 µM) from healthy donors, indicating a degree of selectivity towards malignant B cells. The cytotoxic effects were confirmed to be EP4 receptor-dependent, as they were prevented by an EP4 antagonist and were stronger than those of the endogenous ligand, PGE2. Prostaglandin EP4 receptor agonist PgE1-OH also demonstrated synergistic cytotoxicity when combined with clinically relevant agents, including ibrutinib, idelalisib and venetoclax. Additionally, PgE1-OH altered proinflammatory cytokine production (IL-2, TNFα, IFNγ) in both LCLs and CLL cells, highlighting its immunomodulatory effects, which could further contribute to its therapeutic benefit in the complex CLL microenvironment.

These results collectively identify the prostaglandin EP4 receptor as a promising therapeutic target and PgE1-OH as a potential candidate for combination therapy in CLL treatment strategies.

15. Bridging the Gap Between Chemistry and Oncology for the Breakthrough Application of Fluorescence Probes for Cancer Labelling and Detection

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Fluorescent dyes are indispensable tools for the visualisation of biological systems in science and medicine. Molecules such as fluorescein absorb light at one wavelength and emit it at a longer wavelength – this contrast makes fluorescent areas glow against a dark background. Fluorescein and its derivatives are characterised by their intense fluorescence and adaptability. In medicine, fluorescein is invaluable for procedures such as angiography and fluorescence-assisted surgery, particularly in oncology. In cancer treatment, it improves tumour localisation and reduces damage to healthy tissue. Fluorescein dyes are widely used in bioimaging to distinguish healthy tissue from tumours and histological markers. Their fluorescence helps surgeons to recognise and remove or repair pathological structures.

Recently, some of the structural types of fluorescent markers have been shown to interact successfully with the tumour cell membrane and its physical and chemical

properties. The accumulation of the dye and the mechanisms of activity of the fluorescent molecule depend primarily on the active chemical substituents present on the scaffold of the agent.

All ground-breaking milestones in this field can be attributed to the interdisciplinary cooperation of various specialist areas. In addition to applications, an important segment is the synthesis of new reagents with fluorescence activity, with chemists developing new synthetic strategies and research new analogues resulting from the possibilities of the molecular scaffold. Linking such collaborations with microbiology, immunology, and oncology can thus lead to important findings and their transfer into practice – the treatment of patients – which we as scientists need to strive for.

16. An Unexpected Twist in Inhibiting Cancer Cell Invasion!

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Glioblastoma is a primary brain tumor that is one of the most aggressive and deadliest malignant diseases. Its rapid invasion into surrounding brain tissue makes complete surgical removal nearly impossible, leading to almost certain recurrence. The CD155 protein, a type 1 transmembrane adhesion glycoprotein, is overexpressed on glioblastoma cells. While it normally mediates cell adhesion and contact inhibition, tumor cells hijack its function to support invasion and malignancy. For this reason, a lot of research is focused on inhibiting the function of CD155, thereby improving treatment outcomes. In our study, we tested whether an anti-CD155 antibody (IgCD155) could reduce glioblastoma invasion. First, we determined the concentration of the antibody that could be used without having a toxic effect on cell viability. The MTT assay showed no cytotoxic effects, even at higher doses. We then performed a 3D spheroid invasion assay in Matrigel using a differentiated glioblastoma cell line derived from a patient's tumor biopsy. However, the invasion assay showed that IgCD155 did not affect glioblastoma invasion. Unexpectedly, the control mouse antibody type G1 caused a reduction in invasion, possibly due to binding Fc receptors on the surface of glioblastoma cells. These findings represent the first basis for further studies exploring CD155 as a potential therapeutic target in the treatment of glioblastoma.

17. Spheroid Formation and Characterization by Flow Cytometry Reveal Cancer-Associated Fibroblasts in NSCLC Cultures

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Our aim was to optimize the preparation of viable spheroids of repeatable sizes by centrifugation and to characterize cell cultures by analyzing the expression of cell markers.

We established spheroids from different numbers of cells from cell cultures LC004 and LC005. After optimizing the protocol, we established spheroids from 10,000 cells and then continued their cultivation in ClinoStar bioreactor. The results showed that the overall growth of spheroids was negligible. We noticed reduction in spheroid size and significant cell migration into the medium. Despite those results, spheroids were of reproducible sizes. After the cultivation in ClinoStar, we either observed a shrinkage of spheroids or fusion into irregular clumps.

Afterwards, we characterized both cell cultures and normal fibroblast cell line MRC-5 using flow cytometry. We analyzed the percentage of cells positive for tumor markers cytokeratin 5, cytokeratin 7, p63 and napsin A, the epithelial marker EpCAM, and the fibroblast marker αSMA. The results showed that none of the cell cultures was significantly positive for tumor and epithelial markers, but both strongly expressed the αSMA marker. These results also matched the MRC-5 cell line.

Based on flow results, we concluded that the cultures did not contain cancer cells, but rather cancer-associated fibroblasts (CAFs). This was also consistent with the results of spheroid formation. The shrinkage of spheroids and cell migration into the medium reflects the less pronounced proliferative nature and weaker intercellular contacts of CAFs.

The outcome of the study highlights the problem of CAFs overgrowth and the importance of their exclusion from primary NSCLC cell cultures.

18. The Role of MAGEC2 Protein in Glioblastoma and in Antitumor Immune Response

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Glioblastomas (GBM) are a highly aggressive form of brain tumor, for which there are limited therapeutic options. A significant challenge represent glioblastoma stem-like cells (GSLCs), which are responsible for therapy resistance through rapid self-renewal, robust DNA repair, and effective immune evasion mechanisms. This inherent resilience frequently results in limited efficacy with current treatment modalities, including immunotherapy. However, natural killer (NK) cells offer a promising therapeutic avenue, as they are capable of recognising and destroying these treatment-refractory GSLCs. The present study investigated MAGEC2 (melanoma-associated antigen protein) as a critical factor in GBM resistance and a potential target for combined therapies. MAGEC2 has been demonstrated to be strongly linked to several pro-tumorigenic features, including increased tumor aggressiveness, accelerated proliferation, and genomic instability, suggesting MACEC2 role in enhancing the tumor's resistance and adaptability within its microenvironment. In order to comprehensively investigate its functional impact, we engineered three distinct glioblastoma cell lines to express MAGEC2. Then, we analyzed the impact of this protein on both NK cell-mediated cytotoxicity and the tumor cells' colony-forming ability following radiotherapy. Our results demonstrated that MAGEC2 expression significantly increased radioresistance and altered the cells' sensitivity to NK cell-mediated killing. These findings firmly suggest that MAGEC2 contributes to glioblastoma therapy resilience and warrants further investigation into potential of MAGEC2 in combined therapeutic strategies. Specifically, the targeting of MAGEC2 in conjunction with conventional immunotherapy and radiotherapy has the potential to improve treatment outcomes and enable a more personalised approach glioblastoma patients.

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List of posters

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2	Anamarija	Habič	*National Institute of Biology*	Slovenia
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