PROCEEDINGS BOOK

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PLENARY LECTURES

PL1

The potential of comprehensive liquid biopsy analysis for the management of cancer patients

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Key words: Liquid biopsy, Circulating tumor cells, cell-free DNA, circulating tumor DNA, tumor biomarkers

Over the last decade, liquid biopsy has gained much attention as a powerful tool in personalized medicine since it enables monitoring cancer evolution and follow-up of cancer patients in real time. Through minimally invasive procedures, liquid biopsy provides important information through the analysis of circulating tumour cells (CTCs) and circulating tumour-derived material, such as circulating tumour DNA (ctDNA), circulating miRNAs (cfmiRNAs) and extracellular vehicles (EVs). CTC enumeration and molecular characterization analysis provides important clinical information in patients with many types of cancer. CTC analysis has already had an important impact on the prognosis, detection of minimal residual disease (MRD), treatment selection and monitoring of cancer patientsas this has been clearly shown in many clinical studies. CTC analysis is now an exponentially expanding field in almost all types of solid cancers.
In parallel, plasma cell-free DNA (cfDNA) analysis using a variety of technologies is now established as a liquid biopsy approachto track geneand epigenetic alterations in circulating tumor DNA (ctDNA). ctDNA analysisis highly beneficial for the identification of tumor molecular dynamics and the improvement of personalized treatments for cancer patients. In this presentation, we will discuss whether paired analysis of CTCs and ctDNA provide similar or complementary information for the analysis of liquid biopsy analytes (gene mutations and DNA-methylation markers). We will present data from our direct comparison studies of paired samples of patients with NSCLC, and breast cancer, using the same blood draws and identical downstream molecular assays. Our resultsreveal that CTC-derived gDNAsand paired ctDNA provide complementary information and that CTC-derived gDNA analysis should be further evaluated as an important and complementary tool to ctDNA for guiding individualized therapy.

PL2

ctDNA-Response evaluation criteria in solid tumors - significance for monitoring of patient outcomes

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Keywords: Solid tumors, Liquid biopsies, ctDNA RECIST

This lecture will briefly adress the overall progress in the field of ctDNA research in solid tumors and then engage into the use of ctDNA for monitoring of treatment response in medical oncology and a discussions with focus on the patients perspective.
The development of reliable biomarkers for treatment monitoring is a critical need in oncology. Circulating tumor DNA (ctDNA) has emerged as a minimally invasive, real-time tool for tracking tumor burden and treatment response across solid tumors. While imaging-based treatment evaluation remains the current standard, it provides only delayed and sometimes imperfect correlation with patient outcomes.
To address this gap, the ctDNA-RECIST program has proposed standardized response evaluation criteria based on

quantitative changes in ctDNA levels, derived from droplet digital PCR and validated statistical frameworks. These criteria allow classification of patients into distinct response categories after only one cycle of systemic therapy, with early studies showing strong correlation with progression-free and overall survival.
This lecture will outline the rationale, methodology, and current evidence supporting ctDNA-RECIST, including feasibility data, external validation efforts, and integration with clinical trials. The significance of ctDNA-RECIST for improving monitoring of patient outcomes will be discussed, alongside opportunities for earlier treatment adaptation, reduced toxicity, and optimized use of healthcare resources. Finally, the talk will highlight the ongoing international collaboration to refine, validate, and implement ctDNA-based response evaluation as a complement—or potential alternative—to radiological criteria in daily clinical practice

Acknowledgments and funding: This will be noted at the meeting

INVITED LECTURES

L01

Benefits of Combination Therapies in Cancer Treatment

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Keywords: immunotherapy, radiotherapy, combination therapy, biomarkers, abscopal effect, antibody-drug conjugates, STING agonists

Background: Combination therapies have transformed oncology by overcoming resistance mechanisms seen with monotherapies. Integrating radiotherapy, immunotherapy, targeted therapies, and biomarker-driven personalization has expanded therapeutic options in solid tumors.
Material and Methods: The mechanistic rationale includes immunogenic cell death induced by chemotherapy and radiotherapy, enhancing antigen presentation and T-cell priming. Modulation of the tumor microenvironment through anti-VEGF agents and immune checkpoint inhibitors increases immune infiltration. Additional strategies involve oncolytic viruses, antibody-drug conjugates (ADCs), and innate immune activators such as STING agonists (e.g., MK-1454). Therapeutic pairings include chemotherapy plus immune checkpoint inhibitors (e.g., carboplatin + atezolizumab in NSCLC), targeted therapies with PD-1 blockade (e.g., BRAF/MEK inhibitors + anti-PD-1 in melanoma), and combinations such as ADC + ICI (e.g., enfortumab vedotin + pembrolizumab). AI-guided and multi-omic treatment-matching platforms are increasingly utilized.
Results: Phase III trials show marked survival improvements. IMpower150 reported overall survival (OS) of 19.2 vs. 14.7 months; PACIFIC showed 5-year OS of 43% vs. 29%. CheckMate-067 and 9LA demonstrated long-term survival advantages with combined ICI strategies. In PD-L1+ triple negative breast cancer (TNBC), IMpassion130 indicated clear progression-free survival (PFS) benefit. A representative case involved a 60-year-old male with brain and systemic metastases; following stereotactic radiosurgery and sequential chemo-immunotherapy (cisplatin + pemetrexed → atezolizumab + bevacizumab), complete metabolic response was achieved. Immune-related adverse events (thyroiditis, hepatitis, arthritis) were manageable over 47 cycles of immunotherapy.
Conclusion: Combination therapies represent a new era in oncology. Optimally sequenced and personalized multimodal treatments enhance outcomes. Emerging tools like spatial transcriptomics, microbiome-based prediction, and AI decision platforms will be key for future precision oncology.

L02

Novel Approaches in Radiation Oncology with a Special Focus on Combination Therapies

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Keywords: Chemoradiotherapy, Radiosensitization, Immuno-radiotherapy, UHDR, LaserDriven Particle Beam

1. Combination of radiation-systemic agent

The integration of cytotoxic chemotherapy with radiotherapy has historically been motivated by spatial cooperation and independent cytotoxicity. With growing clinical evidence, chemoradiotherapy has become a standard treatment modality for several malignancies, including glioblastoma, head and neck squamous cell carcinoma (HNSCC), cervical cancer, non–small cell lung cancer (NSCLC), and gastrointestinal tumors. The rationale behind combining chemotherapy with radiotherapy lies in their overlapping and complementary mechanisms. Chemotherapy agents, particularly temozolomide, cisplatin and fluoropyrimidines, act as radiosensitizers by inhibiting DNA repair, synchronizing cell cycles, and increasing oxidative stress within tumor cells [1-3]. Molecular targeted agents interact with RT by inhibiting the repair of radiation-induced double-strand breaks (novel small molecule inhibitors of DNA damage response (e.g., PARP inhibitors) or by interfering with cell cycle checkpoints Notably, inhibitors of ATR, DNA-PK, and CHK1 have demonstrated in vitro and in vivo radiosensitization across multiple cancer models, often yielding ≥2-fold increases in tumor growth delay when administered concomitantly with low LET radiation [4]. Moreover, chemotherapeutic agents can influence the tumor microenvironment to potentiate radiotherapy. For instance, platinum compounds not only induce DNA crosslinks that impede replication but also promote immunogenic cell death, releasing tumor-associated antigens and damage-associated molecular patterns (DAMPs) that activate dendritic cells. Anthracyclines and taxanes similarly trigger immunostimulatory pathways, augmenting local inflammation and cytotoxic T cell infiltration following irradiation. Such immunomodulatory properties lay the groundwork for tripartite combination regimens—chemotherapy, radiotherapy, and immune checkpoint blockade—whereby chemotherapy intensifies radiosensitivity while simultaneously facilitating the generation of an adaptive anti-tumor immune response [5-6]. Optimization of sequencing, dosing, and fractionation remains critical. Personalized regimens guided by tumor genomics and functional imaging biomarkers (e.g., γH2AX PET for DNA damage, FLT PET for proliferation) are under active clinical investigation to refine synergistic potential while safeguarding normal tissues [7].

2. Hypofractionated High Dose Radiation Combined with Immunotherapy

Hypofractionation—delivering larger doses per fraction over fewer sessions is applied in stereotactic body radiotherapy (SBRT) and stereotactic radiosurgery (SRS), achieving local control rates exceeding 90% in select oligometastatic and primary tumor settings due to additional microenvironmental effects. Beyond high local efficacy, high dose fractions induce unique immunogenic effects, including enhanced release of tumor neoantigens, upregulation of type I interferon pathways, and increased expression of MHC class I molecules. These radiation-mediated changes prime the tumor microenvironment for immune checkpoint inhibitors (ICIs), such as anti-PD-1/PD-L1 and anti-CTLA-4 antibodies, promoting immunogenic cell death, augmenting dendritic cell cross-presentation of tumor antigens, and increasing chemokine gradients that recruit effector lymphocytes. Clinically, early-phase trials in non-small cell lung cancer, melanoma, and renal cell carcinoma have demonstrated promising response rates when SBRT is administered to a single lesion followed by systemic ICI therapy, with manageable toxicity profiles. PEMBRO-RT in metastatic NSCLC hasshown improved progression-free survival when pembrolizumab was combined with SBRT. Further prospective studies are now comparing concurrent versus sequential approaches, investigating optimal irradiated volume, fractionation schedules (e.g., 3 × 8 Gy vs. 5 × 6 Gy), and combination partners (e.g., dual checkpoint blockade). Additionally, biomarker research is focusing on circulating tumor DNA, immune cell repertoire dynamics, and spatial transcriptomics to predict responders and tailor treatment intensity.

3. Laser Driven Particle Beams and FLASH Radiotherapy

While combination therapies harness biological synergy, advances in beam physics have led to an increased therapeutic window. The FLASH effect—defined as ultra high dose rate (UHDR) irradiation (≥40 Gy/s) delivered in less than a second—has garnered intense interest due to its remarkable ability to spare normal tissues while retaining tumoricidal efficacy. Conventional accelerator systems face technical and size constraints in achieving UHDRs suitable for clinical FLASH implementation. High-power laser sources, however, offer a novel approach to particle generation and acceleration. Through laser-plasma interactions, electrons, protons, and neutrons can be produced in ultra-short,

high-intensity pulses capable of delivering FLASH-relevant dose rates per pulse at clinically relevant energies. Very High Energy Electron (VHEE) beams generated by laser-driven accelerators further extend this paradigm. VHEEs—in the range of 100–250 MeV—combine deep penetration with precise focusing, potentially enabling conformal dose distributions even in deep-seated tumors. Laser-driven proton beams represent another frontier. Proton therapy's hallmark Bragg peak enables maximal tumor dose deposition with sharp distal fall-off, but conventional cyclotrons and synchrotrons are large and costly. Laser-plasma acceleration may yield compact, cost-effective proton sources capable of UHDR delivery, marrying the spatial selectivity of protons with FLASH sparing.

4. Clinical Integration and Future Directions
Realizing the promise of these novel modalities demands rigorous translational pathways encompassing dosimetric validation, preclinical mechanistic studies, and carefully designed clinical trials. Technological hurdles—such as beam stability, real-time dosimetry at UHDRs, and synchronization with immunotherapeutic dosing—must be addressed. Concurrently, the development of robust biomarkers for normal tissue response and immune activation will guide patient selection and adaptive treatment strategies. Furthermore, computational modeling and artificial intelligence will play pivotal roles in optimizing multi modality schedules, predicting toxicity, and personalizing combination regimens.
In conclusion, the integration of chemotherapy synergistic approaches, hypofractionated high-dose radiation with immunotherapy, and laser-based particle acceleration technologies heralds a new era in radiation oncology. By capitalizing on both physical and biological synergies, these strategies strive to overcome the current therapeutic plateau—offering potent tumor control, diminished radiation damage, and improved patient outcomes.

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L03

Benefits of combination therapies in the treatment of hematological malignancies

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Keywords: hematological malignancies, combination therapy, targeted therapy, benefits

Hematological malignancies, including acute leukemias, and mature lymphoid and myeloid neoplasms, are inherently systemic, involving the bone marrow, blood, and lymphatic tissues from disease onset. Unlike solid tumors, they are not amenable to curative local therapies. Surgery is typically limited to diagnostic procedures or emergency management of complications (e.g., organ rupture, spinal cord compression). At the same time, radiotherapy is reserved for specific indications, including central nervous system (CNS) involvement in high-risk acute leukemia (AL) or aggressive lymphomas, as well as early-stage indolent lymphomas, though it remains adjunctive. Consequently, treatment relies almost exclusively on systemic therapies. The management of hematological malignancies represents one of the most rapidly evolving fields in medical oncology. These cancers arise in blood-forming and lymphoid tissues and involve immune system cells, making them particularly responsive to therapies with cytotoxic and immunomodulatory mechanisms. Over the past century, treatment has progressed from single-agent chemotherapy to rationally designed combination regimens that incorporate chemotherapy, targeted therapies, and immunotherapies, including bispecific antibodies and CAR T-cell therapy, as well as epigenetic modulators. This multifaceted approach has significantly improved response depth and durability, extended survival, and enhanced quality of life, while reducing treatment-related toxicity and long-term morbidity.
Historical perspective and the emergence of combination chemotherapy in hematological malignancies
The origins of chemotherapy lie in observations during the early World War II period, that exposure to mustard gas caused profound lymphoid suppression. This insight was translated into the first clinical use of alkylating agents to treat lymphoma, specifically with the drug mechlorethamine in 1942. The initial successes of these cytotoxic agents, which damage DNA to kill rapidly dividing cells, were limited by the rapid emergence of resistant cancer clones. This limitation spurred the development of combination chemotherapy in the 1950s and 1960s. By using agents with

different mechanisms of action and non-overlapping toxicities simultaneously, the chance of resistant clones surviving was reduced, and responses were improved.

A landmark example of this approach was the MOPP regimen (mechlorethamine, vincristine, procarbazine, prednisone), which dramatically improved outcomes in Hodgkin lymphoma (HL) and became the first chemotherapy combination to cure a human cancer. Subsequently, CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) emerged as the standard treatment for aggressive non-Hodgkin lymphomas (NHL), while in acute myeloid leukemia (AML), the combination of cytarabine and an anthracycline (the "7+3" regimen) set the standard induction therapy. These regimens became the backbone of treatment for decades, laying the groundwork for integrating newer agents.

The advent of targeted therapies and immunotherapy

The therapeutic revolution accelerated in the 1990s and 2000s with the emergence of targeted therapies and immunotherapies. The introduction of rituximab in 1997, an anti-CD20 monoclonal antibody, was pivotal, transforming treatment for B-cell NHL and leading to widespread incorporation of antibody-based therapy in frontline regimens such as R-CHOP. Rituximab's success proved the concept that harnessing the immune system to target specific antigens could augment or replace chemotherapy.

Molecular targeted therapy took a giant leap forward with the development of imatinib in 2001, which specifically inhibits the BCR-ABL fusion kinase that drives chronic myeloid leukemia (CML). Imatinib turned CML into a chronic, manageable disease and demonstrated the feasibility of precision medicine, targeting the cancer's genetic drivers. Since then, numerous targeted agents have been developed for specific genetic and epigenetic abnormalities in hematological malignancies, including FLT3 and IDH inhibitors for AML, BTK inhibitors for mantle cell lymphoma (MCL) and chronic lymphocytic leukemia (CLL).

Integration of novel agents into combination regimens

The current standard of care for many hematological malignancies involves combinations of cytotoxic chemotherapy with targeted agents and immunotherapies to maximize efficacy and overcome resistance. In AML, for example, the integration of FLT3 inhibitors with induction chemotherapy improves survival for patients harboring FLT3 mutations. Similarly, the combination of the BCL-2 inhibitor venetoclax with hypomethylating agents (azacytidine and decitabine) provides a chemotherapy-free induction regimen for older or unfit patients, achieving high complete remission (CR) rates with a more tolerable safety profile.

In multiple myeloma (MM), treatment has shifted away from conventional chemotherapy toward combinations of proteasome inhibitors, immunomodulatory drugs (IMiDs), and monoclonal antibodies. Regimens such as the quadruplet therapy of daratumumab, bortezomib, lenalidomide, and dexamethasone (D-VRd) have become standard in the frontline setting, particularly for transplant-eligible patients. In this population, autologous stem cell transplantation (ASCT) remains an integral component of initial therapy, typically following induction and followed by consolidation and maintenance strategies. These regimens have demonstrated significantly improved progression-free survival (PFS) and overall survival (OS) compared to older chemotherapy-based approaches, with reduced toxicity. Their success is due not only to potent anti-myeloma effects but also to their ability to enhance immune-mediated tumor clearance and achieve high rates of minimal residual disease (MRD) negativity, supporting durable disease control and improved quality of life.

In lymphomas such as HL, novel agents including the antibody-drug conjugate brentuximab vedotin and immune checkpoint inhibitors targeting PD-1 (nivolumab and pembrolizumab) have increased the depth and durability of remission. Notably, these agents have reduced the reliance on salvage regimens followed by ASCT as consolidation for relapsed disease, sparing patients from the morbidity and late effects of high-dose chemotherapy and transplantation. In MCL, incorporation of the BTK inhibitors among other novel agents has improved outcomes in relapsed/refractory settings and increasingly as part of frontline regimens, reducing the necessity for intensive therapies including ASCT in many patients. The less toxic nature of these novel agents allows older or less fit patients to achieve long-lasting remissions.

The significance of complete remission and minimal residual disease

Achieving CR with initial therapy is strongly associated with improved long-term outcomes across hematological malignancies. CR is defined not only by the absence of detectable disease on conventional imaging and bone marrow evaluation but increasingly by molecular assessments of MRD. MRD negativity has emerged as a powerful prognostic marker, correlating with longer DFS and OS in leukemia, lymphoma, and myeloma. Early achievement of deep remission can reduce the need for subsequent intensive therapies such as SCT and decrease the risk of relapse.

Harnessing the tumor microenvironment, chemotherapy-free regimens and improved tolerability

A pivotal advancement underlying many novel therapies is a better understanding of the tumor microenvironment (TME), the surrounding immune cells, stromal cells, blood vessels, and extracellular matrix that interact with and support malignant cells. The TME plays a critical role in promoting tumor growth, mediating immune evasion, and fostering resistance to therapy. Therapeutics like IMiDs and monoclonal antibodies disrupt these supportive interactions and activate immune effector cells. Similarly, checkpoint inhibitors restore exhausted T cells in the TME, reversing tumor-

induced immune suppression. Bispecific antibodies and CAR-T cells represent an innovative way to engage the immune system within the TME by linking T cells directly to tumor cells, bypassing some of the suppressive signals.

The shift toward chemotherapy-free or reduced-intensity regimens involves combining targeted agents, immunotherapies, and epigenetic drugs to deliver effective treatment with reduced toxicity. This translates into fewer hospitalizations, less transfusion dependence, and improved quality of life. The reduction in treatment-related morbidity and late toxicity is critical for long-term survivors, as many hematological malignancies occur in older adults who are particularly vulnerable to the cumulative toxic effects of chemotherapy, including cardiotoxicity, neuropathy, marrow suppression, and secondary malignancies.

Reducing the need for stem cell transplantation

Historically, ASCT has been a cornerstone in consolidating remission in MCL and MM. In relapsed and refractory cases, ASCT has been the standard of care following salvage chemotherapy in chemosensitive NHL patients. While effective, ASCT is associated with significant acute toxicities, prolonged hospitalizations, and long-term risks such as infertility, secondary cancers, and organ dysfunction. The introduction of novel targeted agents and immunotherapies has enabled many patients to achieve durable remissions without ASCT.

Allogeneic stem-cell transplantation (alloSCT) is rarely used in frontline settings but remains an option in selected relapsed or high-risk cases, particularly in AL. Novel therapies are increasingly allowing some patients to delay or avoid transplantation, thereby reducing morbidity and preserving patients' quality of life, without compromising long-term outcomes.

Conclusion

The benefits of combination therapies in hematological malignancies are profound and multifaceted. By harnessing the synergistic effects of cytotoxic chemotherapy, targeted agents, immunotherapies including bispecific antibodies and CAR T cells, and epigenetic drugs, guided by molecular profiling and an improved understanding of the tumor microenvironment, clinicians can achieve deeper remissions, longer survival, and improved quality of life. As research continues to unravel the molecular complexity of hematological malignancies and their interactions with the immune system and TME, future combination therapies are expected to become increasingly personalized, precise, and effective. These advances will not only improve response rates, survival, and quality of life, but also reduce treatment-related toxicity and the need for intensive interventions such as transplantation or prolonged hospitalization. By achieving deeper remissions with less morbidity, these approaches may prove to be more cost-effective, reducing the long-term economic burden on healthcare systems.

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L04

The Critical Role of Tumor Typing in Cancer Treatment and the Expanding Horizons of Molecular Oncology

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Keywords: tumor typing, personalized medicine, molecular oncology, targeted therapy, liquid biopsy

Cancer remains one of the most complex and heterogeneous diseases affecting humanity. Traditional classifications of cancer have relied heavily on histological and anatomical characteristics; however, with the advent of molecular biology and genomics, it has become clear thattumors with a similar appearance under a microscope can behave very differently at the molecular level. This realization has led to the development and growing importance of tumor typing, also referred to as molecular subtyping. Tumor typing involves analyzing the genetic, epigenetic, transcriptomic, and proteomic characteristics of a tumor to betterunderstand its behavior, prognosis, and likely response to specific therapies. This molecular classification has transformed modern oncology, paving the way for personalized and precision medicine, where treatments are tailored to the unique biological profile of each patient's cancer.

Why Tumor Typing Matters in Modern Oncology:

1. Personalized Treatment Approaches
Molecular tumor typing enables the design of patient-specific treatment plans. By identifying mutations, gene expression patterns, or disruptions in signaling pathways driving a particular tumor, oncologists can choose therapies that specifically target these alterations. For instance, in non-small cell lung cancer (NSCLC),tumors with EGFR mutations respond well to EGFR tyrosine kinaseinhibitors (TKIs). Similarly, HER2-positive breast cancers can beeffectively treated with monoclonal antibodies like trastuzumab.

2. Predictive Value for Treatment Response
Not all tumors respond the same way to standard chemotherapy orradiotherapy. Tumor typing allows for the identification of predictivebiomarkers, which indicate how likely a tumor is to respond to certaintreatments. For example, tumors exhibiting microsatellite instability(MSI-H) are more likely to benefit from immune checkpoint inhibitors,whereas those with KRAS mutations in colorectal cancer may not respondto EGFR inhibitors.

3. Prognostic Information
Molecular features of tumors can provide critical prognosticinformation, offering insights into how aggressive a cancer might be, as well as the likelihood of recurrence. Breast cancer subtypes such as Luminal A, Luminal B, HER2-enriched, and Basal-like (triple-negative)have distinct prognoses and clinical behaviors, guiding decisions on the intensity and duration of treatment.

4. Guiding Early Detection and Prevention Understanding the molecular pathways involved in cancer initiation and progression can lead to the development of early detection tools, such as biomarker-based screening tests. For example, the presence of HPV DNA is a key marker in screening for cervical cancer, while circulating tumor DNA (ctDNA) is being explored for early detection of colorectal and lung cancers.

5. Avoiding Overtreatment In some cases, knowledge of a tumor's molecular profile helps clinicians avoid unnecessary aggressive treatments. For instance, in early-stage prostate cancer, identifying low-risk molecular signatures may support a decision for active surveillance rather than immediate surgery or radiation, thereby preserving quality of life.

Expanding Frontiers: Future Perspectives in Molecular Biology of Cancer the future of oncology is inseparably linked to advances in molecular biology. As our ability to analyze tumors at the molecular level improves, new tools and therapeutic approaches are emerging that hold the promise of transforming cancer care.

1. Liquid Biopsy and Non-Invasive Diagnostics
One of the most exciting advancements is the development of liquid biopsy, which involves detecting cancer-related biomarkers in bodyfluids, primarily blood. By analyzing circulating tumor cells (CTCs), ctDNA, or exosomes, liquid

biopsies can offer insights into tumor dynamics without the need for invasive tissue sampling. This techniqueis particularly useful for monitoring treatment response, detecting minimal residual disease, and identifying emerging resistance mutations.

2. Immunotherapy and Immune Profiling
Immunotherapy represents a paradigm shift in cancer treatment. Therapies such as immune checkpoint inhibitors (e.g., PD-1/PD-L1blockers) have shown remarkable success in cancers like melanoma, lung cancer, and bladder cancer. However, not all patients benefit. Ongoing research is focused on identifying immune-related biomarkers, such as tumor mutational burden (TMB) and PD-L1 expression levels, to predict which patients are most likely to respond. In the future, tumor immune microenvironment profiling may become standard practice in guiding immunotherapy decisions.

3. Targeted Therapies and Precision Oncology
Targeted therapies aim to block the activity of molecules essential for tumor survival and growth. The success of agents like imatinib in chronic myeloid leukemia (targeting BCR-ABL fusion protein) and vemurafenib in BRAF-mutant melanoma illustrates the power of this approach. The ongoing development of next-generation sequencing (NGS) technologies is enabling the identification of rare but actionable mutations, allowing patients with previously untreatable cancers to receive effective, targeted drugs.

4. Epigenetic Therapies and Gene Editing
Alterations in DNA methylation, histone modification, and non-coding RNA expression play crucial roles in cancer. New therapeutic strategies aim to reverse these changes using epigenetic drugs, suchas DNA methyltransferase inhibitors or histone deacetylase inhibitors. In addition, CRISPR-Cas9 gene editing technology is being explored to directly correct genetic defects or disrupt oncogenes, although clinical application is still in the early stages.

5. Artificial Intelligence (AI) and Big Data in Cancer Genomics
With the explosion of genomic and clinical data, AI is becoming an essential tool in oncology. Machine learning algorithms can analyze complex molecular data to predict disease progression, treatment response, and survival outcomes. AI is also accelerating drug discovery by identifying novel therapeutic targets based on large-scale cancer genome datasets.

6. Multi-Omics Integration
A single type of data, such as genomics alone, is often insufficient to fully capture the complexity of cancer. Therefore, integrating multi-omics approaches—including genomics, transcriptomics, proteomics, and metabolomics—is becoming increasingly important. This comprehensive view enables researchers to uncover novel pathways and interactions that contribute to tumor behavior, opening doors to more effective combination therapies.

7. Combination Therapy Strategies
Cancer cells often develop resistance to monotherapies. As a result, combining therapies with different mechanisms of action is gain ingtraction. For example, combining immune checkpoint inhibitors with targeted therapies or chemotherapy has improved survival in several cancers. Rationally designed combination regimens based on tumor biology are expected to enhance efficacy and overcome resistance.

Challenges and Ethical Considerations. Despite the promise of molecular oncology, several challenges remain. Tumor heterogeneity, both between patients (intertumoral) and within a single tumor (intratumoral), complicates diagnosis and treatment. Additionally, access to high-end molecular testing is often limited by cost and infrastructure, especially in low-resource settings. Ethical issues also arise, particularly with genomic testing. The discovery of incidental findings—mutations unrelated to the current cancer but relevant to future health—raises questions about disclosure and genetic counseling. Data privacy and informed consent are also critical concerns in the age of precision medicine.

Conclusion. Tumor typing is not just a scientific advancement—it is a clinical necessity. By revealing the molecular intricacies of each tumor, it guides the selection of targeted, effective therapies and paves the way for truly personalized cancer care. As molecular biology tools continue to evolve, we move closer to an era in which cancer treatment is guided not by where the tumor originated, but by the unique molecular fingerprint it carries. From non-invasive diagnostics and immunotherapies to AI-powered genomic analysis and multi-omics integration, the future of oncologyis one of precision, personalization, and possibility. Continued investment in research, technology, and equitable access will be key to ensuring that these innovations benefit all cancer patients, regardless of geography or background.

L05

Manipulation of genes involved in both drug resistance and drug-induced EMT reveals unpredictable ovarian cancer cell morphology and stress response

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Keywords: ovarian cancer, drug resistance, metastasis, TMEM200A, PRKAR1B

In the treatment of ovarian cancer (OC), even initially responding patients eventually develop drug resistance. Previously, TMEM200A and PRKAR1B were recognized as potentially involved in the drug resistance and the metastatic potential of OC. Furthermore, we have shown that both genes have high predictive and prognostic value for OC patients. The aim of further work is to investigate the signaling pathways behind the TMEM200A- and PRKAR1B-mediated regulation of the above-mentioned phenomena. For this purpose, OC cells with stable overexpression of TMEM200A or PRKAR1B products as well as cells with knock-out of TMEM200A and PRKAR1B were established and characterized. The data obtained so far show that the influence on drug resistance and metastatic potential mediated by TMEM200A and PRKAR1B depends on their expression level in the cell and the origin of the cell line. Further work on newly established research models is needed to understand how TMEM200A and PRKAR1B are involved in drug resistance and drug-induced metastatic potential of OC.

L06

Repurposing antidiabetic drugs as a therapeutic approach for breast cancer

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Keywords: breast cancer, antitumor immune response, antidiabetic drugs, metformin, semaglutide

Background: Breast cancer is the second most frequent type of malignancy in the female population with more than 2.3 million cases and 666,000 deaths in 2022. Triple-negative breast cancer occurs in about 12%-17% of all breast cancer patients with tendency for early recurrence and fast progression, leading to metastasis in distant organs. Given the fact that diabetes and cancer share several metabolic pathways, drug repurposing is shedding light on the use of antidiabetic drugs against various types of cancers. Our main goal was to examine how antidiabetic drugs, metformin and semaglutide, affect breast cancer growth and antitumor immune response.
Material and Methods: BALB/C wild type female mice were injected with 4T1 breast cancer cells and subsequently treated with metformin and semaglutide.
Results: Metformin delayed tumor growth via stimulation of function of NK, NKT cells and T cells and inhibition of Tregs and MDSCs. Metformin administration significantly increased the expression of immunostimulatory miRNA-150 and miRNA-155, while it decreased the expression of immunosuppressive miRNA-146a in isolated NK cells. Semaglutide application slowed down tumor appearance, growth and progression. In comparison to metformin, semaglutide promoted antitumor immune response in non-NK cell way, by increasing accumulation of CD11c+ dendritic cells, while decreasing Tregs in spleen and primary tumor. Furthermore, semaglutide enhanced the cytotoxic capacity of CD8+ T cells in vitro.
Conclusion: Metformin and semaglutide target different components of antitumor immune response thereby efficiently reducing breast cancer growth.

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L07

Cancer-associated fibroblast spatial heterogeneity

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Keywords: tumor-suppressing CAF, single cell RNA sequencing, therapy resistance

Metastatic dissemination is responsible for over 90% of cancer-related deaths worldwide. The liver serves as a major hub for metastases from some of the most lethal malignancies, including colorectal, breast, lung, and pancreatic cancers. More than 50% of patients with advanced colorectal cancer (CRC) will develop liver metastases (CRC-LM) within five years following resection of the primary tumor. Of these patients, only one-third are eligible for surgical intervention; the remainder must rely on systemic, chemotherapy-based treatments. Although primary tumors, such as CRC, can often be effectively resected, metastatic disease remains largely incurable. Chemotherapy, with or without targeted therapies, frequently leads to the development of resistance and tumor progression. These treatments lack specificity for cancer cells, resulting in considerable toxicity to normal tissues. Consequently, chemotherapy cannot be administered at doses sufficient to eradicate the tumor. Due to these limitations, survival for CRC-LM patients is generally poor—median overall survival is approximately five years for operable patients and only two years for those who are inoperable. It is now well-established that therapy-driven selection of drug-resistant clones is a key contributor to treatment failure (Diaz et al. Nature 2012). Genomic sequencing studies have revealed profound genetic heterogeneity among cancer cells within a single tumor (Gerlinger et al. NEJM 2012). Beyond mutational diversity, the tumor microenvironment is a major determinant of disease progression. It is widely acknowledged that cancer cells and the surrounding stroma form a complex ecosystem, whose collective fitness dictates natural or therapy-driven selection (Polyak et al. Trends Genet. 2009). Furthermore, a critical yet often overlooked trait of such aggressive cancer cells is their capacity to interact with and exploit host stromal tissue to access key resources like growth factors and metabolites (Hunttila& De Sauvage, Nature 2013). Despite the recognized importance of the tumor microenvironment, we still lack a comprehensive understanding of how cancer and stromal cells interact and how this interplay can be therapeutically disrupted. Promising clinical results with immune checkpoint inhibitors (e.g., PD-L1 blockade) illustrate that interrupting cancer–microenvironment communication can yield substantial benefits. However, only a subset of patients currently benefits from these therapies, highlighting the need to explore other stromal components beyond immune cells. The stromal compartment includes fibroblasts, endothelial cells, macrophages, and various immune cells. Among them, cancer-associated fibroblasts (CAF) represent the most dynamic and multifunctional population, implicated in virtually all hallmarks of cancer (Ronca et al. CurrOpin Oncol. 2018). CAFs are known to secrete a wide array of growth factors, extracellular matrix proteins, immune modulators, and energy substrates. In advanced tumors, CAFs are typically "educated" to co-evolve with cancer cells, supporting tumor progression and resistance to therapy. For instance, hepatocyte growth factor produced by fibroblasts can rescue melanoma cells harboring mutant BRAF from BRAF inhibition (Straussman et al. Nature 2012), while fibroblast-derived PDGF-C has been shown to counteract anti-VEGFA therapy in murine lymphomas (Crawford et al. Cancer Cell 2009). Importantly, this pro-tumorigenic activity of fibroblasts is not their default behavior. On the contrary, fibroblasts are naturally programmed to restrain tumor growth (Dotto et al. PNAS 1988; Proia & Kuperwasser, Cell Cycle 2005). Emerging evidence suggests that CAFs exhibit both tumor-promoting and tumor-suppressive roles (Chiavarina&Turtoi, Curr Med Chem 2017). Just as macrophages can polarize into M1-like (tumor-suppressive) or M2-like (tumor-promoting) states, recent single-cell studies have confirmed that CAFs are not a homogeneous population. Notably, these anti-tumor CAF functions appear to persist even in advanced tumors, as demonstrated in pancreatic cancer (Özdemir et al. Cancer Cell 2014; Rhim et al. Cancer Cell 2014). Advances in single-cell technologies have led to the identification of multiple CAF subtypes in primary colorectal and breast tumors (Li et al. Nat Genet. 2017; Costa et al. Cancer Cell 2018). In breast cancer, certain CAF subpopulations have been directly linked to an immunosuppressive phenotype, thereby contributing to tumor progression (Costa et al. Cancer Cell 2018). These foundational studies support the long-suspected heterogeneity of CAFs. However, whether similar CAF subpopulations exist in metastatic lesions—and what roles they play—remains an open question. Another

unresolved issue is how aggressive cancer cells circumvent the initial suppressive nature of fibroblasts to ultimately convert them into tumor-supporting CAFs. The identity of the cancer-derived factors that mediate this reprogramming is still largely unknown. Our own work has shown that metastatic breast cancer cells, unlike their non-aggressive counterparts, secrete IL-1β, which binds to the IL-1 receptor on CAFs and suppresses the secretion of asporin (ASPN) (Maris et al. PLOS Med. 2015). ASPN is a potent inhibitor of TGF-β1, a key driver of epithelial-to-mesenchymal transition (EMT) and stemness in cancer cells. Similarly, Ijichi et al. reported that pancreatic cancer cell-derived CXCL1 induces CTGF expression in CAFs to promote tumor growth (Ijichi et al. J Clin Invest. 2011). In yet another recent study, our group has reported in hepatocellular carcinoma that tumor-suppressive CAF can secrete prolargin (PRELP) which can bind and inhibit a set of key growth factors that promote tumor progression (Chiavarina et al. Oncogene 2022). These findings, while illuminating, represent only a fraction of the complex cancer–stroma interactions, and many more remain to be discovered—particularly in the context of specific tumor types and therapy resistance. The failure of indiscriminate CAF targeting using Sonic hedgehog (SHH)-smoothened (SMO) signaling inhibitors in clinical trials (Catenacci et al. JCO 2015) underscores the need for a more nuanced understanding of CAF function. Collectively, these insights call for a paradigm shift in how we study and therapeutically exploit the tumor stroma, especially under the selective pressures imposed by cancer treatments. To this end, our lab has recently embarked on a combination of spatial and single-cell omics in multiple cancers including liver metastases. This approach permits for the first time a better understanding of CAF tumor heterogeneity, offering additional means to differentiate tumor promoting versus tumor suppressing CAF-derived molecules (Giguelay et al. Theranostics 2022, Honda et al. Theranostics 2024).

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L08

Understanding Factors Influencing Immunotherapy Response in Head and Neck Cancer

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Keywords: head and neck cancer; CD8+ T cells; clinical trial; single-cell genomics; T cell dynamics; combination immunotherapy

Background: In the evolving landscape of immuno-oncology, a key unresolved question is whether different immune checkpoint inhibitors (ICI) and their combinations promote similar immune mechanisms during a favorable treatment response, or whether they act through distinct pathways. To address this critical gap in knowledge, we leveraged a novel phase II neoadjuvant clinical trial (NCT04080804) designed to enhance adaptive anti-tumor immunity in patients with resectable, locally advanced head and neck squamous cell carcinoma (HNSCC). Patients were treated with anti-PD-1 (nivolumab; Nivo) monotherapy, or in combination with either anti-LAG-3 (relatlimab; Nivo+Rela), a regimen previously untested in this disease, or with anti-CTLA-4 (ipilimumab; Nivo+Ipi) monoclonal antibodies.
Materials and Methods: Forty-one patients were randomized across the three treatment arms. To evaluate treatment-induced immune mechanisms, we performed extensive single-cell analyses using pre- and post-treatment matched tumor specimens from 35 patients. Single-cell RNA sequencing, T cell receptor (TCR) sequencing, and Cellular Indexing of Transcriptomes and Epitopes by Sequencing (CITE-seq) were performed on 372,914 $CD45^+CD3^+$ tumor-infiltrating lymphocytes (TIL), including 137,133 $CD8^+$ TIL. Multiplex immunofluorescence imaging was used to quantify $CD8^+$ T cell infiltration and its association with pathologic response.
Results: Both Nivo+Ipi and Nivo+Rela combination therapies elicited superior pathologic responses compared to

Nivo monotherapy. Pathologic response in the Nivo+Rela arm correlated with increased CD8⁺ T cell infiltration post-treatment. Nivo+Rela treatment expanded and reprogrammed exhausted CD8⁺ TIL characterized by type I interferon and exhaustion gene signatures into effector and tissue-resident memory (TEM/TRM) phenotypes. In contrast, Nivo+Ipi expanded pre-existing TEM/TRM CD8⁺ TIL without rejuvenating exhausted cells. CD8⁺ TCR diversity increased in major responders to Nivo+Rela but not to Nivo+Ipi, and both combination regimens demonstrated minimal clonal replacement, suggesting a reliance on baseline TIL pools.
Conclusions: Despite engaging distinct CD8⁺ TIL subsets and transcriptional programs, both Nivo+Rela and Nivo+Ipi ultimately promoted functional TEM/TRM populations. These findings reveal regimen-specific immune mechanisms underlying successful neoadjuvant ICI responses in HNSCC and inform rational design of future immunotherapeutic strategies.

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L09

Dual blockage of PD-L/PD-1 and IL33/ST2 axes as a potential antitumor therapy

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Keywords: breast cancer, antitumor immunity, PD1, IL-33

Background: Despite great advances in early diagnosis and treatment, breast cancer is still the leading cause of death among women aged 35 to 54 years of age. Although radiotherapy, chemotherapy and surgery are options for treating breast cancer, there has been an increase in the exploration of alternative treatments, such as immunotherapy. Our goal was to examine how modulation of antitumor immunity affects breast tumor growth and progression. Although separate blockage of either IL33/ST2 or PD-L/PD-1 axes has been shown to be beneficial in many tumors, co-blockage of IL33/ST2 and PD-L/PD-1 has not been studied yet.
Material and Methods: 4T1 breast cancer and CT26 colon cancer were induced in BALB/C wild type (WT) and BALB/C ST2 knockout mice, after which mice underwent anti PD-1 and anti IL-33 treatments.
Results: Co-blockage of IL33/ST2 and PD-L/PD-1 delayed tumor appearance and slowed tumor growth. Enhanced NK cell cytotoxicity was associated with overexpression of miRNA-150 and miRNA-155, upregulation of NFκB and STAT3, increased expression of activation markers and decreased expression of immunosuppressive markers in splenic and primary tumor-derived NK cells. Accumulation of immunosuppressive myeloid-derived suppressor cells and regulatory T cells was significantly impaired in the spleen and primary tumor of ST2 knockout anti-PD-1-treated mice.
Conclusions: Co-blockage of IL3/ST2 and PD-L/PD-1 axes impedes tumor progression more efficiently than the single blockage of either axis, thus offering a potential new approach to tumor immunotherapy.
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L10

Immune Checkpoint Inhibitors Modulate Cystatin F Expression in Cytotoxic T Cells from Melanoma Patients

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Keywords: melanoma, cystatins, immune checkpoint inhibitors, cytotoxic T lymphocytes

Background: Despite their superior clinical efficacy over conventional chemotherapy and radiotherapy, immune checkpoint inhibitors (ICIs) remain ineffective in over 60% of cancer patients. Moreover, cytotoxic immune cells targeting tumors can also damage healthy tissue, leading to potentially life-threatening adverse events. These limitations underscore the urgent need for early, reliable predictive biomarkers to improve patient management and reduce treatment costs. Even among initial responders, only a minority experience durable benefit, largely due to diverse resistance mechanisms—particularly in solid tumors, where the immunosuppressive tumor microenvironment (TME) plays a central role. Additionally, dysfunctional CTL effector activity following release from checkpoint blockade further limits tumor cell killing and therapeutic efficacy. A deeper understanding of resistance pathways and CTL dysfunction is critical for the development of effective combination strategies aimed at enhancing ICI responses. Therapies that target complementary immune escape mechanisms may yield synergistic effects and improve clinical outcomes. Insights into CTL impairment may also guide optimization of other immunotherapies, including CAR T cell therapy. Emerging biomarkers could help identify the most effective T cell subsets for adoptive cell transfer in combination with ICIs. Cytotoxic T lymphocytes (CTLs) eliminate cancer cells by releasing cytotoxic molecules—such as the pore-forming protein perforin and apoptosis-inducing granzymes—into the immunological synapse between the effector and target cell. These effector molecules are synthesized as inactive precursors and require activation via proteolytic processing by cysteine cathepsins. Cystatin F, an endogenous inhibitor of these cathepsins, has been identified as a potent negative regulator of CTL cytotoxic function. In natural killer (NK) cells, which share the same granule-mediated cytotoxic pathway, reduced killing capacity—termed “split anergy”—is linked to increased cystatin F and decreased levels of active granzyme convertases (cathepsins C and H). Similarly, cystatin F expression is elevated in CTLs rendered anergic by ionomycin or TGF-β, correlating with diminished cytotoxicity. Ex vivo long-term stimulation of $CD4^+$ T cells also induces expression of granzymes A and B, cathepsins C and H, perforin, and cystatin F. We further established a model using PD-1–expressing cytotoxic TALL-104 cells, in which preliminary data show that anti–PD-1 antibody treatment leads to upregulation of cystatin F, suggesting a link between ICI therapy and modulation of CTL effector function. Cystatin F is initially synthesized as a disulfide-linked dimer, which is inactive as a cathepsin inhibitor. Following synthesis, it is directed to the endo/lysosomal compartment via the mannose-6-phosphate (M6P) pathway, facilitated by multiple N-linked glycans. Within endo/lysosomes, cystatin F undergoes proteolytic cleavage by the cysteine peptidase cathepsin V, converting it into its active form. In this monomeric state, cystatin F potently inhibits lysosomal peptidases, specifically cathepsins C and H. Its ability to translocate to endo/lysosomes—essential for activation—is influenced by the type of N-glycosylation it undergoes. Our recent unpublished findings reveal that the glycosylation profile of cystatin F shifts from high-mannose to complex-type N-glycosylation in NK cells with differing cytotoxic capacities, including NK-92 cells, primary NK cells, and IL-2–activated “super-charged” NK cells. These results suggest that modulation of cystatin F glycosylation or its interaction with M6PR can affect its trafficking and subsequent activation. Since only the monomeric form of cystatin F inhibits cathepsins C, H, and L within cytotoxic granules, targeting the activation process represents a promising strategy to restore cytotoxic function.
Our recently published work supports this concept: treatment with a small-molecule inhibitor of cathepsin V (compound 7) reduced cystatin F monomerization and activation, leading to enhanced cytotoxicity in NK-92 cells. Furthermore, cathepsin V inhibition increased granule-mediated killing by primary NK cells against stem-like glioma cells (NCH421k), underscoring the therapeutic potential of this approach. Cystatin F expression has been shown to increase in the tissue of certain tumors as the disease progresses. Survival analysis of RNA sequencing data from the TCGA dataset, including patients with low-grade gliomas and grade IV glioblastoma, indicates that higher cystatin F expression correlates with shorter overall survival. This finding is particularly significant given that both the monomeric and dimeric active forms of cystatin F can be internalized by immune effector cells through the mannose-6-phosphate receptor (M6PR) pathway, targeting the endo/lysosomal compartment. Uptake of extracellular cystatin F has been demonstrated to reduce the susceptibility of glioblastoma cells to NK cell–mediated cytotoxicity. Additionally, internalization of both forms of

cystatin F by NK cells leads to a marked reduction in their cytotoxic function by decreasing the activity of effector molecules granzymes A and B. Similar efficient uptake of extracellular monomeric and dimeric cystatin F has been observed in primary CTLs and the $CD8^+$ T cell line TALL-104. Once internalized, cystatin F localizes to cytotoxic granules, where it co-localizes with granzyme A and perforin, and its primary binding partner has been identified as cathepsin C. This interaction ultimately results in reduced cytotoxic activity in both primary human CTLs and TALL-104 cells.

Material and Methods: To comprehensively investigate the role of cystatin F in the context of immune checkpoint inhibitor (ICI) therapy, we analyzed its expression in cytotoxic T lymphocytes (CTLs) derived from peripheral blood mononuclear cells (PBMCs) and evaluated its presence in tumor tissues collected prior to treatment initiation. Tumor samples were used to examine the localization and abundance of cystatin F-expressing cells and to explore potential relationships with the immune microenvironment. In parallel, we conducted an in silico analysis of publicly available transcriptomic datasets to assess cystatin F expression patterns across tumor types, its prognostic significance, and its correlation with immune cell infiltration signatures. Peripheral blood samples were collected from melanoma patients undergoing first-line ICI therapy—nivolumab, ipilimumab/nivolumab, or pembrolizumab—at the Institute of Oncology Ljubljana, Slovenia. Samples were obtained at three defined timepoints: 4 weeks prior to therapy, and 12 ± 2 and 28 ± 2 weeks following treatment initiation. Longitudinal profiling of cystatin F expression in CTLs was performed at each timepoint to capture dynamic changes during treatment. Patients were clinically monitored for up to 12 months post-treatment initiation and stratified into responder and non-responder groups based on RECIST criteria and overall clinical outcome. PBMCs were isolated by density gradient centrifugation, cryopreserved, and subsequently thawed for downstream analysis. $CD8^+$ T cells were enriched using magnetic-activated cell sorting (MACS). Flow cytometry was employed to quantify intracellular cystatin F protein expression in CTLs, while quantitative real-time PCR (qRT-PCR) was used to measure transcript levels of cystatin F and cytolytic effector genes including perforin and granzymes A and B. In addition, SDS-PAGE and western blot analyses were conducted to assess the expression and activation state of key cytotoxic granule components. This multimodal approach enabled us to link molecular and functional profiles of CTLs with clinical response to ICI therapy and to evaluate the utility of cystatin F as a predictive biomarker of therapeutic efficacy.

Results and Conclusions: Our longitudinal analysis demonstrated that cystatin F expression in cytotoxic T lymphocytes (CTLs) declined progressively in melanoma patients who responded to immune checkpoint inhibitor (ICI) therapy. This temporal decrease was most pronounced in individuals achieving durable clinical responses, suggesting that elevated cystatin F expression may be associated with impaired CTL function, while its downregulation could reflect restored or enhanced cytolytic activity during effective treatment. These findings support the potential role of cystatin F as a dynamic biomarker of immunotherapy response, particularly in the context of $CD8^+$ T cell-mediated tumor clearance. Although subtle differences in cystatin F kinetics were noted between male and female patients, these observations require validation in larger cohorts and were not the primary focus of this investigation. Taken together, our results underscore the clinical relevance of monitoring cystatin F expression in peripheral CTLs during the course of ICI therapy. As a regulator of effector function in cytotoxic immune cells, cystatin F may serve as a useful indicator of therapeutic efficacy and disease trajectory in melanoma patients. Incorporating cystatin F into existing biomarker panels could enhance patient stratification and aid in tailoring treatment strategies, thereby improving outcomes while minimizing unnecessary exposure to ineffective therapies. Future studies should focus on validating these findings across broader patient populations and further elucidating the molecular mechanisms underlying cystatin F modulation in the setting of immune checkpoint blockade.

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L11

Multidrug resistance – poly-specific drug recognition by ABC transporters

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Keywords: ATP-Binding Cassette (ABC) transporters; multidrug resistance; P-glycoprotein (Pgp; ABCB1)

ABC transporters such as ABCB1 and ABCG2 are active exporter proteins expressed in the plasma membrane of cells forming biological barriers. In accordance with their broad substrate spectrum and tissue expression pattern, they affect the pharmacokinetics of numerous chemotherapeutic drugs and they may be involved in unwanted drug-drug interactions leading to side effects or toxicities. When expressed in tumor tissues, they contribute to the development of chemotherapy resistance in malignancies. Therefore, the understanding of the molecular details of the ligand-ABC transporter interactions is of crucial importance. In our previous studies we identified numerous compounds of diverse origin and chemical nature as ABCB1 substrates or inhibitors using ATPase activity measurements, transport assays, and exploiting the UIC2 conformation-selective monoclonal antibody (mAb), that recognizes the inward-facing (IF) conformer of ABCB1. Our measurements demonstrated that the most potent transport inhibitors strongly inhibit ATPase activity and stabilize ABCB1 in the UIC2-reactive IF conformation. In a recent study, we analyzed the interaction of numerous polyphenols with ABCB1. We found that quercetin (QUR) hampers both the transport and ATPase activity of ABCB1, while cyandin-3O-sophroside (C3S) stimulates the ATPase activity and causes only a weak inhibition of substrate transport. When QUR and C3S were applied together, both a stronger ATPase inhibition and a robust decrease in substrate transport were observed, supporting their synergistic ABCB1 inhibitory effect. Similar to cyclosporine A, a potent ABCB1 inhibitor, co-treatment with QUR and C3S shifted the conformational equilibrium to the IF conformer of ABCB1. To gain deeper insight into the molecular details of ligand-ABCB1 interactions, molecular docking experiments and MD simulations were also carried out. Our in silico studies confirmed that QUR and C3S can bind simultaneously to the substrate-binding pocket of ABCB1. The C3S-QUR combination formed a larger number of hydrogen bonds with higher occupancy involving the amino acid side chains of the substrate-binding pocket compared to the simultaneous binding of two QUR molecules, thereby contributing to the conformational stabilization and stronger inhibition of ABCB1.

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L12

Eliminating Oral Cancer Stem Cells by Targeting Oncogenic miR-21 and BET-Mediated Transcription

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Keywords: Cancer stem cells; Epigenetics; Oral cancer; microRNA.

Background: Oral squamous cell carcinoma (OSCC) remains the most widespread and aggressive form of head and neck cancer, with cancer stem cells (CSCs)—identified by CD44 expression—contributing significantly to its progression, therapeutic resistance, and recurrence. MicroRNA-21 (miR-21), a well-characterized oncogenic microRNA (miR), is implicated in promoting chemoresistance and poor patient prognosis across multiple tumor types.
Material and Methods: In this study, we explored the impact of miR-21 suppression on CD44+ CSCs in the context of treatment with the BET bromodomain inhibitor JQ1. CD44+ subpopulations were isolated from OSCC cell lines,

followed by targeted miR-21 silencing and pharmacological treatment with JQ1.
Results: The combined intervention resulted in enhanced JQ1 sensitivity, marked by increased apoptotic activity, reduced invasive potential, and cell cycle disruption. Molecular analysis revealed that these effects were mediated through modulation of apoptotic regulators and components of the WNT signaling cascade.
Conclusions: Our data highlight a potent synergistic interaction between miR-21 inhibition and BET targeting, suggesting a novel combinatorial approach to eliminate CSCs and improve therapeutic efficacy in OSCC.

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L13

The role of UFMylationin head and neck cancer stem cells

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Keywords: neoplasms, proteomics, therapeutics

Background: Head and neck squamous cell carcinoma (HNSCC) is the seventh most common cancer, associated with tobacco and alcohol use or viral infections with human papilloma virus (HPV) or Epstein-Barr virus (EBV). The recurrence and treatment resistance, commonly seen in head and neck squamous cell carcinoma (HNSCC), may be due to cancer stem cells (CSCs). Tumors contain a subpopulation of cells called cancer stem cells (CSCs). These cells have the unique abilities to self-renew, differentiate, and initiate new tumors, and they are often linked to resistance to treatments like chemotherapy and radiotherapy. Both the tumor microenvironment and genetic alterations can drive CSC plasticity.CSC markers include CD44, CD133, CD24, CD90, and aldehyde dehydrogenase1 (ALDH1). Cancer cells acquire these stem-like traits through epithelial-mesenchymal transition (EMT), a process driven by transcription factors like Twist1/2, ZEB1/2, and Snai1/2 (1).
The UFMylation pathway is similar to ubiquitination, regulating a variety of biological processes by post-translationally attaching to proteins. Ubiquitin Fold Modifier- 1 (UFM1) is a ubiquitin-like modifier that is post-translationally attached to lysine residues on substrates through a system of enzymes conserved in most eukaryotes: Ubiquitin Like Modifier Activating Enzyme 5 (UBA5), Ubiquitin-Fold Modifier Conjugating Enzyme 1 (UFC1), and UFM1 Specific Ligase 1 (UFL1) classified as the E1, E2, and E3 enzymes respectively, while UFM1 Specific Peptidase 1 (UFSP1) and UFM1 Specific Peptidase 2 (UFSP2) are the UFM1-specific proteases.It is important to note the reversibility of UFMylation: UFSPs are capable of cleaving UFM1 from the substrate protein, thereby enabling the recycling of UFM1 and its corresponding substrates. Despite associations between mutations in UFM1 pathway genes and various diseases, the functional importance of UFMylation is still unclear. It is known that UFMylation is associated with ER homeostasis, DNA damage response, p53 stabilization, protein biogenesis and regulation, autophagy etc. Researchers are still trying to understand UFMylation's involvement in cancer, as studies have yielded contradictory findings depending on the specific cancer type. A large number of amplifications and deletions of genes encoding UFMylationproteins have been found in tumors (2).Toll-like receptors (TLRs) are a key component of the immune system. These evolutionarily conserved pattern recognition receptors (PRRs) recognize molecular patterns originating from pathogens, subsequently activating immune responses. TLR3 binds dsRNA or its synthetic analogs poly (I:C) and poly (A:U) (3).The role of TLR3 in cancer is still not clear: some studies suggest it could be a target for cancer therapy as its activation leads to apoptosis (4), but other studies reveal its unfavorable role resulting inthe promotion of tumor progression by either metabolic reprogramming (5), or increased angiogenesis and migration (5, 6). Additionally, one study so far has associated TLR3 activation with stem cell-like phenotypes in breast cancer cells (7), and we have recently published that TLR3 activation promotes stemness in HNSCC lines (8).
Our proteomic analysis of CSCs, followed by further investigations, shows that UFMylation likely plays a significant role in HNSCC CSCs.
Materials and Methods: HNSCC-derived cancer cell lines Detroit 562, FaDu, and Cal27, and tumor spheres were used as a model for CSCs research. Tumor spheres were produced by cultivation of the cells in MEBM™ Mammary Epithelial Cell Growth Basal Medium (Lonza), supplemented with B-27™ Supplement (ThermoFisher Scientific), 20 ng/ mL of Epidermal growth factor (EGF), and 10 ng/mL of Fibroblast growth factor (FGF) (Peprotech, UK) in low adhesion dishes.

Proteomic analysis performed by Biocentar (Zagreb, Croatia) identified several proteins involved in the UFMylation pathway in CSCs. This was further studied by bioinformatics, transient transfection and RNA silencing, western blot, immunocytochemistry, RNA isolation, real time PCR and cytotoxicity assay.The RNA and proteins were isolated from tissue of 43 HNSCC patients admitted to the Clinical Hospital Dubrava (Zagreb, Croatia) and collected during surgery by a Mini kit for RNA and protein purification (Macherey-Nagel, Dueren, Germany). Procedures that followed were conducted according to the ethical protocols approved by the Institutional Ethics Committee. Informed patient consent was obtained prior to surgery.

Results: We have performed proteomic analysis on 4 sets of samples: adherent cells, untreated tumor spheres, tumor spheres treated with poly (I:C), and tumor spheres treated with poly (A:U). We found 51 changed proteins in tumors spheres compared to adherent cells, 46 changed proteins in tumor spheres treated with poly (A:U) compared to untreated tumor spheres, and 22 changed proteins in poly (I: C)-treated tumor spheres compared to the untreated tumor spheres. By using STRING analysis, we have determined many different protein interactions. However, in this study we have focused on the UFMylation pathway, as several of the changed proteins belonged to this pathway: UFSP2 and DDRGK1 as enzymes involved in this system, and RPL9 and RPL29 as target proteins of UFMylation. We have first confirmed our proteomic analysis results by western blot. Given the observed overexpression of UFSP2 in tumor spheres when compared to adherent cells, we proceeded with a more in-depth investigation of this phenomenon. Bioinformatic analysis revealed that high expression of UFM1 is linked with worse overall and disease-free survival, and it correlated with the main EMT proteins in HNSCC. Our experiments showed that UFM1 was also strongly expressed in tumor spheres compared to the adherent cells.Its inhibition by siRNA reduced tumor sphere growth, downregulated CD133, and also reduced the expression of EMT markers fibronectin and vimentin. Our bioinformatic analysis, using FunRich, showed that the Sp1 transcription factor regulates nearly 50% of the proteins we found altered in our proteomic study. Therefore, we investigated mithramycin, Sp1 inhibitor, as a potential therapeutic agent targeting specifically CSCs. We have established that mithramycin reduced cell survival of three HNSCC lines Detroit 562, FaDu and Cal27, through apoptosis induction as evidenced by PARP cleavage. The UFMylation process was disrupted in mithramycin-treated tumor spheres after 72 h, as there is almost no visible UFM1 and UFM1 conjugates expression.Mithramycin also reduced the expression of other UFMylation proteins DDRGK1, UBA5, and UFC1, but also the expression of RPL26, which is a target for UFMylation (9). Finally, we have analyzed HNSCC patients tissue samples to establish the level of expression of UFSP2 and DDRGK1.

Conclusions: Our findings indicate that UFMylation is crucial for HNSCC cancer stem cells (CSCs). We propose that inhibiting UFM1 or other UFMylation pathway proteins (DDRGK1, UBA5, UFC1) could be a reliable strategy to delay HNSCC progression by targeting CSCs. Such inhibition is achievable with Sp1 inhibitors like mithramycin or its analogs. This research highlights UFM1's potential as a prognostic biomarker and a therapeutic target for HNSCC, specifically in eliminating CSCs through apoptosis.

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L14

Common, low-penetrance genetic predisposition to colorectal cancer

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Keywords: colorectal cancer, genetic predisposition, cancer risk, genetic variant, genetic association

Colorectal cancer (CRC) is recognized as one of the cancers with the highest incidence and associated mortality worldwide [1]. It is generally acknowledged that a vast majority of CRC cases develop from non-malignant precursor adenomas [2]. The average duration of the development of an adenoma to CRC transition is unobserved, but it is estimated to take at least 10 years [3]. This long latent phase provides an excellent window of opportunity for early detection. Therefore, CRC is particularly suitable for screening. Keeping in mind the magnitude of this disease, European national health systems have started population screening programs in order to increase early detection and improve prevention measures. Screening for CRC offers the possibility to identify the disease at an earlier stage or at a premalignant phase. For this reason, the evidence-based European Code Against Cancer recommended that men and women over 50 years of age should participate in CRC screening. This was given effect within the EU by the 2003 Council Recommendation on cancer screening [4].

Indeed, CRC is highly preventable by detecting and removing adenomas through colonoscopy screening, but this procedure is very costly to be implemented as population screening and has an associated morbidity [5]. Intermediate screens to detect occult blood in feces such as fecal immunochemical testing (FIT) are therefore often used to select patients for colonoscopy with suboptimal sensitivity [6, 7]. This 2-step strategy for CRC screening is the most common worldwide [8], but results in a high false positive rate due to the suboptimal specificity of the occult blood detection, implying unnecessary colonoscopies [9]. Therefore, additional biomarkers added to the first step to the current scenario could improve CRC screening.

As for other complex diseases, CRC is caused by both genetic and environmental factors [10]. Twin studies showed that around 13%-30% of the variation in CRC susceptibility involves inherited genetic differences [11, 12]. Some of the known CRC predisposition factors were already discovered in the past two decades through genome-wide association studies [13, 14]. Right after their identification, the hope was raised for genetic pro-filing using the combination of these common, low-penetrance genetic variants to be able to identify high-risk individuals in the population that could benefit from preventive and therapeutic interventions [15]. Indeed, polygenic risk scores (PRS) combining the individual, weak effects on disease risk have been developed in the past for common diseases such as CRC. Their predicting potential was limited, most likely evidencing their usefulness but their shortcomings when used alone without other clinical or environmental data [16, 17]. PRS models for CRC were developed by using individual genome-wide association study (GWAS) genetic variants (from 10 to more than 100) but have recently incorporated genome-wide data in order to improve risk prediction [18]. Certainly, genome-wide PRS have proven to identify individuals with risk equivalent to monogenic mutations [19], which could justify its application in health care systems.

Using PRS to screen the population at medium risk for CRC is an attractive alternative to improve current results in this setting. Frampton et al. demonstrated that personalized screening programs for CRC, in which eligibility was based on PRS in addition to age, had the potential to greatly reduce the number of individuals screened while still detecting nearly as many cases [20]. Some more recent studies have also tested the potential application of PRS on CRC screening programs, showing its value to define a personalized, risk-adapted starting ages for screening [17, 21] or personalized screening intervals after negative findings from colonoscopy [22].

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L15

Adjuvant therapy options for patients with breast cancer and germline BRCA1/2 mutations

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Keywords: BRCA 1/2 mutations, breast cancer, PARP inhibitors

Backround: Breast cancer is a global health problem and the most common cancer in women in both resource-rich and resource-limited settings. The lifetime probability of developing invasive breast cancer is approximately one in eight. It is a heterogeneous, phenotypically diverse disease composed of several biologic subtypes that have distinct behaviors and responses to therapy.
The use of adjuvant systemic therapy is responsible for much of the reduction in cause-specific mortality from breast cancer (1).
Pathogenic variants in BRCA1 and BRCA2 are associated with significantly elevated lifetime risks of breast, ovarian, pancreatic, and prostate cancer (2,3). These genes are critical in double-strand break repair through homologous recombination. An understanding of the biology of BRCA1 and BRCA2 led to the development of targeted therapeutics, specifically poly(ADP-ribose) polymerase (PARP) inhibitors, which have been approved by the US Food and Drug Administration for multiple BRCA1/2-associated cancers.

Main findings
The development of poly(ADP-ribose) polymerase inhibitors (PARPi) for BRCA1/2 mutation carriers has been a major advance in targeted therapeutics for tumor suppressor genes by deploying the concept of synthetic lethality. Synthetic lethality refers to cell death with inhibition or gene alteration of two DNA damage response pathways, but not with alteration in either pathway alone. Synthetic lethality allows for the targeting of tumor suppressor genes with the potential for limited toxicity in heterozygous cells. In 2005, two seminal studies were published demonstrating that synthetic lethality could be leveraged in BRCA1/2-deficient tumors: cells deficient in homologous recombination (HR) were killed when PARP was inhibited (4,5). Clinical efforts followed, and the phase I trial of the PARPi olaparib was published in 2009, which demonstrated objective responses in advanced breast, ovarian, and prostate cancer patients with gBRCA1/2 (6). Subsequent studies led to the regulatory approval of multiple PARPi (i.e., olaparib, niraparib, rucaparib, and talazoparib) for the treatment of gBRCA1/2-associated breast, pancreatic, prostate, and ovarian cancer.

Early HER2 negative breast cancer, adjuvant therapy
A randomized, double-blind trial, OlympiA, was conducted in 1836 patients with high-risk, HER2-negative early breast cancer with BRCA1 or BRCA2 pathogenic variants and high-risk clinicopathologic factors who had received local treatment and neoadjuvant or adjuvant chemotherapy (7).
High risk features, according to the OlympiA eligibility criteria, are the following:

a. for the triple-negative breast cancer (TNBC) treated with neoadjuvant chemotherapy patient must have residual disease to be eligbe for adjuvant olaparib, while for patients treated with adjuvant chemotherapy, to have higher-risk features patient must to have either node-positive disease or a primary tumor ≥2 cm on the surgical specimen;
b. for the hormone receptor-positive disease treated with neoadjuvant chemotherapy patients with residual disease and a CPS+EG≥3 (the pretreatment clinical stage and post-treatment pathologic stage, as well as estrogen receptor status and tumor grade, CPS+EG score) were considered as eligable, while for patients treated with adjuvant chemotherapy high risk was considered as of N2 disease.

Olaparib treatament was given for one year at the dose of 300 mg twice daily, orally.
In this trial, patients assigned to the olaparib group had an improvement in three-year DFS relative to the placebo group (86 vs 77%; difference, 8.8%; 95% CI 4.5-13.0); distant DFS at three years was 88% in the olaparib group and 80% in the placebo group (difference, 7.1%; 95% CI 3.0-11.1); four-year overall survival was 90 vs 86% (HR 0.68, 98.5% CI 0.47-0.97) (8). The benefit of adjuvant olaparib relative to placebo was observed for invasive DFS irrespective of the germline BRCA mutation (BRCA1 vs BRCA2), hormone receptor status, carboplatin administration, or whether chemotherapy was administered in the neoadjuvant versus adjuvant setting.
These data justify genetic testing for all patients meeting the OlympiA eligibility criteria. Olaparib has regulatory approval by the US Food and Drug Administration for the adjuvant treatment of adult patients with deleterious germline BRCA-mutated, HER2-negative, high-risk early breast cancer who have been treated with neoadjuvant or adjuvant chemotherapy.

Discussion

The OlympiA trial demonstrated that one year of adjuvant olaparib can significantly reduce the risk of recurrence and help prevent progression to metastatic disease in patients with high-risk early breast cancer who carry germline BRCA1 or BRCA2 pathogenic variants. This treatment was associated with high adherence rates and primarily low-grade toxicity.

As awareness grows regarding the impact of germline BRCA1 or BRCA2 pathogenic or likely pathogenic variants on treatment decisions, an increasing number of these variants are being identified in patients with early breast cancer.

The development of PARP inhibitors (PARPi) for BRCA1/2-associated cancers represents a major advancement in cancer therapy. The hope is that by identifying which tumors are most likely to develop resistance to PARPi and understanding the key mechanisms to prevent or reverse such resistance, whether through new therapies or novel combinations—treatment outcomes will improve.

Timing may be crucial, as evidenced by the OlympiA trial in BRCA-associated breast cancer and by first-line maintenance therapy in ovarian cancer. Ongoing studies are also exploring neoadjuvant approaches. Furthermore, improving assays for detecting homologous recombination deficiency (HRD) and deepening our understanding of predictors of PARPi response beyond just germline and somatic pathogenic variants are essential steps toward expanding the patient population that can benefit from these therapeutic strategies.

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L16

Exploring the Metabolic Landscape of IDH-Mutant Gliomas for Therapeutic Intervention

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Keywords: glioma, metabolism, inflammation, methylation

Background: Glioblastoma multiforme (GBM) – the most malignant of brain cancers, characterized by an aberrant metabolic profile, is largely refractory to current therapeutic regimens. Mutations in isocitrate dehydrogenase (IDH), – a rate-limiting enzyme in TCA cycle that catalyses the conversion of isocitrate to α-ketoglutarate (α-KG), are prominent in lower-grade gliomas and secondary glioblastomas. Mutations in the IDH1 gene are associated with a better clinical outcome when compared to patients harboring gliomas with the wild-type IDH1 gene. The common point mutation R132H in IDH1 (IDH1-R132H) is associated with a gain-of-function activity that converts α-KG to d-2-hydroxyglutarate (d-2HG), which accumulates in IDH1 mutant gliomas [1]. IDH1 mutation establishes CIMP (CpG island methylator phenotype) by remodeling the methylome and transcriptome, resulting in distinct methylation and transcription patterns in mutants as compared to IDH1WT [2]. We have previously reported that IDH1 mutation in gliomas alters redox balance [3, 4], chemotherapeutic resistance [5], and immune evasion pathways [6]. 2-HG, produced in IDH1-MT, functionally alters the metabolism of glioma cells. As the rewiring of metabolic reprogramming with the epigenetic landscape in gliomas can differentially influence chemoresistance, we investigated whether the altered metabolic profile of tumours harboring IDH1 mutation contributes to the differential methylation status of genes associated with survival responses and chemoresistance in these tumours.
Materials and Methods: Genome-wide methylation on tumors derived from IDH1 wild-type and mutant glioma patients was performed using Infinium Methylation EPIC v2.0. Immunohistochemistry, quantitative real-time PCR (qRT-PCR), Western blot analysis, were performed on patient derived tumor samples harboring IDH-MT or WT counterparts. In vitro genetic and pharmacological manipulations, together with biochemical assays, were conducted on commercially available glioma cell lines, treated with 2HG to mimic IDH1MT conditions or stably or transiently transfected with IDH1-WT and IDH1-R132H constructs as described previously [6].
Results: Methylation profiling data from IDH wild-type and mutant glioma patients indicated hypermethylation of several significant regulators of inflammation, including the suppressor of cytokine signaling 3 (SOCS3) in IDH1MT. This finding in clinical samples was recapitulated in cells harboring IDH1 mutation. In vitro genetic and pharmacological studies indicated SOCS3 as a molecular target of glycolysis, as inhibition of glycolysis affected SOCS3 expression through altered methylation at its promoter. Given the substantial prognostic relevance of SOCS3 and its potential as a target for immunotherapy, we identified drugs from Genomics of Drug Sensitivity in Cancer (GDSC) [7], directed at clinically impactful genes related to the glycolysis-SOCS3 axis. The identified drug, which is a known anti-cancer chemotherapy target, induced death in both IDH1-MT and IDH1-WT cells. This heightened sensitivity to drug targeting the glycolysis-SOCS3 axis was SOCS3-dependent, as SOCS3 over-expression in IDH1MT cells rescued the cytotoxic effect.
Conclusions: Our findings suggest that the distinct genetic landscape rewires energy metabolism and survival responses in the context of distinct molecular signatures associated with predictive and prognostic values in glioma patients. By understanding the less aggressive phenotypes of IDH1 mutant gliomas and exploring various cell survival and death pathways, we can gain novel insights into exploiting these signaling molecules for their therapeutic significance.

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L17

The (first) 20 years of BRCA testing in Croatia

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Keywords: Breast neoplasms, Genetic Techniques, Genetic Testing, Hereditary Breast and Ovarian Cancer Syndrome, Mutation, Ovarian neoplasms

Background: Hereditary Breast and Ovarian Cancer syndrome is primarily caused by inherited mutations in *BRCA1* and *BRCA2* genes.
Patients and Methods: To establish a robust and quick detection method for *BRCA1* and *BRCA2* screening, healthy women, over the age of 65, with no family history of cancer were tested for *BRCA1* and *BRCA2* variants to determine the frequencies of harmless variants. 220 samples were screened for *BRCA1* and 115 for *BRCA2*. Subsequently, 647 patients were tested for presence of deleterious mutations. Healthy controls and first 260 samples were analyzed by HRM (high resolution melting) and Sanger sequencing for point mutations and QMPSF (quantitative multiplex PCR of short fluorescent fragments) and LR-PCR (long-range PCR) for large rearrangements. The next 387 samples were analyzed by NGS (next generation sequencing) and QMPSF, and results confirmed by Sanger sequencing and LR-PCR.
Results: Thirteen different *BRCA1* mutations were found in 52 patients and fifteen different *BRCA2* mutations were found in 23 patients. Most common mutations were *BRCA1* c.5266dupC (p.Gln1756fs) found in fifteen patients, *BRCA1* c.181T>G (p.Cys61Gly) found in eleven patients, *BRCA2* c.6641dupC (p.Tyr2215fs) found in eight patients and *BRCA1* c.1252G>T (p.Glu418Ter) found in seven patients. In *BRCA1* one large deletion of exons 4-6 was found in three non-related patients, while in *BRCA2* two large deletions were found in two patients, deletion of whole *BRCA2* in one and exons 1 and 2 in second.
Conclusions In Croatia: *BRCA1* mutations are more frequent than *BRCA2* mutations, with *BRCA1* c.5266dupC being the most common mutation. Large rearrangements are not common. Using HRM and QMPFS, or NGS and QMPFS to screen for mutations and Sanger sequencing for confirmation are superior combination of methods for optimum results.

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L18

The Importance of Homologous Recombination Repair Deficiency (HRD) in Ovarian Cancer

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Keywords: homologous recombination repair deficiency (HRD), genomic instability, HR proficiency

Homologous recombination repair deficiency (HRD) has emerged as a pivotal biomarker and therapeutic target in the management of ovarian cancer, particularly high-grade serous ovarian carcinoma (HGSOC), which constitutes the most common and lethal subtype. HRD reflects an impaired ability of tumor cells to accurately repair DNA double-strand breaks via the homologous recombination (HR) pathway, leading to genomic instability and tumorigenesis. This deficiency is frequently caused by deleterious mutations in key HR pathway genes, such as BRCA1 and BRCA2, but can also result from epigenetic silencing or other genetic alterations affecting the broader HR repair machinery. Understanding the role of HRD in ovarian cancer has significantly advanced the landscape of precision oncology. Tumors exhibiting HRD demonstrate increased sensitivity to DNA-damaging agents, including platinum-based chemotherapies and, more recently, PARP (poly ADP-ribose polymerase) inhibitors, which exploit synthetic lethality to selectively target HR-deficient cancer cells. Consequently, HRD status has become an essential criterion in treatment stratification, guiding therapeutic decisions and improving clinical outcomes.

The identification of HRD involves both direct genetic testing for BRCA mutations and comprehensive genomic assays that detect loss of heterozygosity (LOH), telomeric allelic imbalance (TAI), and large-scale state transitions (LST), which are indicative of a broader "HRD phenotype." Despite its clinical utility, challenges remain in standardizing HRD testing, interpreting results, and addressing resistance mechanisms that can emerge during PARP inhibitor therapy. This abstract underscores the critical importance of HRD in ovarian cancer from both a biological and clinical perspective. It highlights the need for continued research into refining HRD diagnostics, understanding tumor heterogeneity, and developing novel therapeutic strategies that can extend the benefit of HR-directed therapies to a wider patient population. As our knowledge deepens, HRD assessment is likely to become increasingly central to the personalized treatment paradigm in ovarian cancer care.

Homologous recombination repair deficiency (HRD) is a critical molecular vulnerability in ovarian cancer, particularly in high-grade serous ovarian carcinoma (HGSOC), the most prevalent and aggressive subtype. HRD impairs the ability of cells to repair DNA double-strand breaks via the high-fidelity homologous recombination (HR) pathway. This leads to genomic instability, accumulation of mutations, and accelerated tumor evolution. HRD can result from germline or somatic mutations in BRCA1 and BRCA2, but also from epigenetic silencing or defects in other HR-related genes such as RAD51C, RAD51D, or PALB2. As such, HRD is not only a hallmark of ovarian cancer biology but also a determinant of therapeutic response.

The importance of HRD in ovarian cancer lies in its dual role as both a prognostic and predictive biomarker. Patients with HRD-positive tumors—particularly those with BRCA1/2 mutations—tend to exhibit improved responses to platinum-based chemotherapy, the mainstay of first-line treatment. More significantly, HRD status predicts profound benefit from PARP inhibitor therapy, which has revolutionized the treatment paradigm. Agents such as olaparib, niraparib, and rucaparib exploit synthetic lethality by selectively killing HRD-positive tumor cells while sparing normal tissue, thereby improving progression-free survival and delaying disease recurrence.

L19

Novel MiRNA Signatures as Potential Biomarkers for Circulating Tumor Cell Status in Metastatic Colorectal Cancer

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Keywords: biomarker; circulating tumor cells; metastatic colorectal cancer; microRNA; microarray.

Background: Colorectal cancer (CRC) is one of the most prevalent cancers and the second leading cause of cancer-related deaths worldwide. Recent studies highlight the promising role of microRNAs (miRNAs) in predicting circulating tumor cell (CTC) status. Combining miRNA and CTC analyses may offer a powerful approach to improving metastatic cancer risk assessment. This study identified circulating miRNA signatures linked to CTC status in metastatic CRC.
Material and Methods: A total of 48 mCRC patients and 50 healthy individuals (HIs) were recruited. The CTC evaluation was performed using AdnaTestColonCancer technology. Total RNA was isolated from whole blood, and miRNA expression profiles were determined with the Agilent miRNA Microarray platform. Three miRNAs (hsa-miR-199a-5p, hsa-miR-326, and hsa-miR-500b-5p) that were dysregulated (FC≥2 or ≤-2 and $p<0.05$) in CTC-positive and CTC-negative individuals were analyzed through quantitative PCR using the miRCURY LNA miRNA PCR system. The diagnostic accuracy of these miRNAs as potential biomarkers for distinguishing CTC-positive, CTC-negative, and HIs groups was evaluated by the ROC analysis.
Results: The analyzed miRNAs exhibited statistically significant downregulation ($p<0.05$) in CTC-positive patients compared to CTC-negative patients and HIs. ROC analyses revealed the area under the curve (AUC) values above 0.7, suggesting their effectiveness in distinguishing the CTC status of mCRC patients. Additionally, these miRNAs were significant in the TCGA-COAD (Colon Adenocarcinoma) and TCGA-READ (Rectum Adenocarcinoma) RNA-Seq datasets and associated with CRC in the Ingenuity Pathway Analysis.
Conclusion: Our findings highlight the potential utility of circulating miRNAs as a complementary method for identifying the CTC status in mCRC. These results also provide new insights for new translational medicine applications in managing mCRC through miRNA-based strategies for CRC-associated CTC detection.

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L20

Bridging Cancer Care and Research Gaps in Croatia: The CORN-CCI4EU Deep Dive

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Cancer remains a leading cause of death in Europe, and Croatia faces persistent regional disparities in access to comprehensive oncology services. The EU-funded **CORN-CCI4EU Deep Dive**, launched under the CCI4EU horizon project, aims to address these inequities by supporting the development of Comprehensive Cancer Infrastructures (CCIs) in Croatia. The project's goal is that by 2030, at least 90% of patients who could benefit from multidisciplinary

cancer care will have equitable access, regardless of their geographic location.
A core component of the initiative is the integration of research into clinical care. Through capacity-building programs, healthcare professionals are gaining enhanced skills to improve infrastructure for clinical research, data-driven decision-making, and evidence-based practice. Participating centers are encouraged to collaborate with academic institutions, increase patient participation in clinical trials, and include translational research outcomes into everyday oncology workflows.
To further enhance system-wide quality, CORN-CCI4EU supports the standardization and optimization of patient pathways through utilization of real-world data and outcome indicators. Activities include comprehensive site assessments, regional educational events, and implementation of secure data-sharing platforms for benchmarking and knowledge exchange.
The CORN-CCI4EU Deep Dive exemplifies the transformative potential of EU-supported collaboration: aligning clinical practice with research, reducing regional inequalities, and investing in expertise and infrastructure. Early insights and best practices from the project underline its role in shaping a sustainable, innovation-driven cancer care model.

L21

CircRNA-miRNA interactions and bioinformatics analysis in cancer

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Keywords:cancer, CircRNA, miRNA

Although only a small part of the human genome-approximately 2%-consists of protein-coding genes, the remaining 98% comprises non-coding sequences. As research into these non-coding regions expands, the critical roles of non-coding RNAs (ncRNAs) in gene transcription and expression regulation have become increasingly apparent [1]. Once dismissed as “junk DNA,” ncRNAs have become a central focus in cancer biology and molecular research. Their newly discovered roles offer promising avenues for cancer diagnosis and prognosis, underscoring their potential as valuable biomarkers and therapeutic targets [2]. Advances in next-generation RNA sequencing and sophisticated analytical techniques have revealed that circular RNAs (circRNAs) and microRNAs (miRNAs) are integral components of the ncRNA family [3].
CircRNAs are a class of endogenous ncRNAs characterized by their covalently closed-loop structure, which lacks both 5' caps and 3' poly-A tails. This unique circular conformation makes them resistant to exonuclease degradation, conferring exceptional stability. Among the well-documented functional mechanisms of circRNAs are their ability to act as molecular sponges for miRNAs, their interactions with RNA-binding proteins (RBPs), their potential to encode proteins, and their regulatory influence on gene expression at both the transcriptional and post-transcriptional levels [4]. Recent scientific advances have highlighted one of the most prominent roles of circRNAs: their miRNA-sponging activity, which inhibits the functional impact of miRNAs [5]. miRNAs, typically 19–23 nucleotides in length, are small, single-stranded, ncRNA molecules that play crucial roles in the post-transcriptional regulation of gene expression [1]. Highly conserved across species and exhibiting tissue-specific expression patterns in humans, miRNAs are essential for regulating key biological processes such as apoptosis, cellular proliferation, stress responses, and differentiation. CircRNAs selectively contain varying types and quantities of miRNA response elements (MREs), enabling them to inhibit miRNA activity and consequently upregulate the expression of miRNA-associated target genes. Additionally, circRNAs can transiently store or transport miRNAs, thereby influencing the expression patterns of genes regulated by these miRNAs. Acting as endogenous miRNA sponges, circRNAs can function as oncogenes or tumor suppressors, depending on the context of their molecular interactions [5].
Tumor bioinformatics plays an important role in cancer research and its support in this field has become indispensable. Large-scale cancer datasets have rapidly accumulated with the advancement of high-throughput technologies in recent years [6]. Bioinformatics approaches are increasingly utilized to elucidate the pathophysiological mechanisms underlying diseases and to identify pharmacological targets at both the genetic and proteomic levels [7]. The integration and analysis of these data resources provide valuable insights into the molecular pathways driving tumorigenesis. Moreover, such analyses facilitate the identification of early diagnostic and prognostic biomarkers and support the development of personalized therapeutic strategies [6].
Several online databases support predictive analysis of circRNA–miRNA interactions, including CircRNAprofiler, Circ2GO, CircNet 2.0, CircAtlas 3.0, and updated versions of CircInteractome [8].

Given the significant roles that both circRNAs and miRNAs play in various biological processes, accurately characterizing the potential interactions between these two classes of ncRNAs is essential. Such insights enhance our understanding of disease mechanisms and contribute meaningfully to the diagnosis, treatment, and prognosis of various pathological conditions. Furthermore, these molecules hold promise as future therapeutic targets or agents, particularly in cancer therapy. Ultimately, elucidating the complex network of circRNA-miRNA interactions may facilitate innovative advancements in cancer therapy and customized treatment. In conclusion, investigating circRNA-miRNA interactions via sophisticated bioinformatics methods is crucial for enhancing our comprehension of cancer biology and utilizing these findings in clinical applications.

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L22

Proteomic and metabolomics profiling of advanced melanoma patients to predict and monitor the therapeutic response to immune therapy

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Keywords: melanoma, biomarker, immune therapy, proteomics

Background: Despite the high clinical need, there are currently no biomarkers in practice that can accurately predict or monitor the response of patients with metastatic melanoma to anti-PD-1 therapy. Recently, the analysis of non-invasively obtained finger sweat has been postulated as a promising method for identifying markers for chronic inflammation or for inferring changes in tumor metabolism.
Materials and Methods: Serum samples were collected before anti-PD1 immune therapy. Finger sweat is always collected before and after treatment at the first time point of therapy, at 3 weeks (2nd cycle of immune therapy) and at 3 months after treatment. The relevant clinical data were recorded. The samples obtained were analysed by liquid chromatography-mass spectrometry using an Orbitrap Exploris 480 and timsTOF mass spectrometry. Subsequent bioinformatics analysis was performed.
Results: In our most recent publication, we created a marker signature with 10 key serum markers (CRP, LYVE1, SAA2, C1RL, CFHR3, LBP, LDHB, S100A8, S100A9 and SAA1) that could indicate a poor response to anti-PD-1 therapy in melanoma patients. Here, we wanted to apply a completely new, non-invasive method in which eccrine sweat from the fingertips is collected using a special paper and metabolites are determined from it. We were able to show that, for example, markers of tryptophan metabolism such as kynurenine, markers of dysbiosis in the microbiome such as p-cresol sulphate, and markers of mitochondrial stress are upregulated in the finger sweat of melanoma patients and

could be relevant parameters for monitoring during immunotherapy. In the next step, these and new markers will be evaluated in the larger cohort at the three different time points.
Conclusions: This comprehensive study could enable us to identify new predictive and pharmacodynamic biomarkers for immunotherapy at the metabolite level. This offers new opportunities for patient stratification, early detection of non-responders and side effects, and further understanding of resistance. By using this new innovative and non-invasive method, we can capture processes in patients without major thresholds.

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L23

Personalized Genomic Analysis for Clinical Insights in Non-Small Cell Lung Cancer

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Keywords: Exome Sequencing, Molecular profiling, NSCLC, Patient-Derived Cell Cultures, Personalized Therapy

Background: Non-small cell lung carcinoma (NSCLC) accounts for approximately 85% of all lung cancer cases and remains the leading cause of cancer-related mortality worldwide [1]. Clinical treatment has improved significantly in recent years with the introduction of targeted therapies and immune checkpoint inhibitors. Therapeutic decisions are largely guided by the identification of somatic genetic driver alterations in tumors [2]. However, this approach alone is often insufficient to capture the complexity of tumor biology and functional response to treatment.
Decreasing sequencing costs and advances in the analysis of sequencing data have opened up the possibility of using whole-exome sequencing (WES) in the clinic. Applying this approach to genomic profiling enables the identification of all potential driver mutations, tumor mutational burden, homologous recombination deficiency, mismatch repair deficiency, copy number alterations, mutational signatures and even germline information for each patient [3]. By overcoming the limitations of targeted panels, WES facilitates more personalized characterization of tumor biology, which is important for optimizing precision oncology approaches [4,5]. In parallel, functional testing of the patient-derived tumor cells in response to drugs offers a direct way of assessing treatment sensitivity [6]. Drug testing on primary cell cultures, including chemotherapeutics and tyrosine kinase inhibitors (TKIs), enables real-time assessment of cytotoxicity and treatment sensitivity that can be directly correlated with the underlying genomic landscape [7].
By integrating functional drug response data with WES-based genomic profiling, our approach aims to provide a potentially comprehensive diagnostic platform for NSCLC. This combined methodology not only identifies clinically actionable genomic features, but also validates their functional relevance, thus improving predictive power. It also allows us to identify rare, exceptional responders in unselected patients, which is not possible with current patient selection strategies. Ultimately, this integrative approach represents a step towards truly personalized cancer diagnostics, where both the molecular and functional characteristics of the tumor are taken into account in clinical decision-making.
Methodology: *Collection of NSCLC tissue samples.* Tissue samples from 67 NSCLC patients were collected from the Clinic for Thoracic Surgery at the University Clinical Center of Serbia with informed consent obtained from the patients and approved by the Ethics Committee of the University Clinical Center of Serbia (approval reference number 623/4). Samples were collected during surgery and histopathologically examined to confirm the diagnosis of NSCLC, histologic grade, stage, necrosis and status of lymph node invasion. Fresh tumor tissue destined for primary cell culture was placed in sterile tubes containing an antibiotic–antimycotic solution and immediately transported to the research laboratory for further processing. For WES, paired tumor and corresponding normal tissue samples were frozen in liquid nitrogen immediately after surgical removal and stored in liquid nitrogen until DNA isolation.
Patent-derived NSCLC cell cultures. Patient-derived NSCLC cell cultures containing both cancer cells and stromal components were established from freshly collected NSCLC tumor samples. These primary cultures were maintained

in vitro for 1 – 2 weeks to ensure cell attachment, growth and stabilization prior to drug testing.
Fluorescence immunoassay. An immunofluorescence assay was developed to screen patient-derived NSCLC cell cultures for their response to chemotherapeutics and TKIs. Drug concentrations were selected based on clinically relevant exposure levels, with the maximum plasma concentration (Cmax) as the upper limit and four lower concentrations to capture dose-dependent effects. Automated microscopy (ImageXpress Pico, Molecular Devices, San Jose, CA, USA) and image analysis with CellReporterXpress software were used to quantify total cell number and differentiate cancer cells from stromal cells based on cytokeratin (CK8/18) expression. The cytotoxicity was determined using the Cell Scoring Analysis Protocol, which evaluates changes in the viability of cancer cells under different treatment conditions.
WES. Genomic DNA was extracted from paired tumor and corresponding normal tissue samples from NSCLC patients using the QIAamp Fast DNA Tissue Kit. Sequencing was performed by Novogene Co (Cambridge, UK). Library preparation was performed using the SureSelect Human All Exon V6 Capture Kit, and paired-end 150 bp reads were generated on the Illumina NovaSeq 6000 platform. At Novogene, raw sequencing reads were subjected to initial quality control using fastp (version 0.23.1), clean reads were aligned to the human reference genome (GRCh37) using the BWA-MEM algorithm (version 0.7.8-r455), and post-alignment processing was performed with the Picard toolkit (version 2.6.0). The processed BAM files containing high quality aligned reads were provided to our team. Subsequent bioinformatic analyses were performed in-house using a pipeline developed in R/Bioconductor and shell-based tools. Somatic single nucleotide variants (SNVs) and small insertions/deletions (InDels) were identified using VarScan2 (version 2.4.3), with tumor normal pair analysis allowing accurate discrimination between somatic events and germline polymorphisms. For germline variant detection, the GATK HaplotypeCaller (version 4.2) was used to generate high-confidence germline variant call sets. Structural variants (SVs) were detected using Manta, which identifies genomic rearrangements such as translocations, inversions and large insertions/deletions. Copy number variations (CNVs) were profiled using CNVkit, which provides genome-wide CNV profiles at exome resolution. PureCN was used to determine tumor purity and ploidy as well as allele-specific copy number states. Variant annotation was performed with the Variant Effect Predictor (VEP) (ensembl-vep-release-105), which integrates several annotation resources, including RefSeq, dbSNP, COSMIC, ClinVar, 1000 Genomes, NHLBI-ESP, gnomAD, SIFT, PolyPhen, and HGMD-PUBLIC. This annotation workflow provided detailed information on the functional consequences of each variant, pathogenicity predictions and clinical relevance. Analysis of mutational signatures was performed using the R package signature.tools.lib, which decomposes the observed mutational spectra into known COSMIC signatures for single base substitutions (SBS), double base substitutions (DBS) and indel signatures (ID) and detects potential novel mutational processes active in the tumors. All results were integrated, curated and analyzed using R (v4.3) and Bioconductor. Somatic and germline variants, CNV profiles and mutational signatures were summarized with custom scripts and visualized with tools such as maftools and circlize. The Cell Scoring Analysis Protocol described above was combined with the WES results to identify potential correlations between genetic alterations and drug response.
Results: To investigate the therapeutic response of NSCLC patient cells, we performed ex vivo drug testing with a range of chemotherapeutic agents and TKIs currently used in clinical practice. The cell response of NSCLC patients was assessed using a modified scoring system previously described [8], which integrates multiple weighted parameters to provide an overall drug response score. This approach allowed quantitative comparison of drug effects on cancer and stromal cells as assessed by immunofluorescence-based cell classification. The analysis revealed considerable variability in sensitivity profiles between patients, with some drugs showing strong cytotoxic effects in certain cultures while being ineffective in others.
To complement the ex vivo drug sensitivity testing and gain deeper insight into the molecular features that influence treatment response, we performed WES of tumor and corresponding normal tissue in each NSCLC patient. This genomic profiling allowed us to identify somatic mutations, germline variants, copy number alterations and mutational signatures that may underlie the differences in therapeutic sensitivity observed in the patient-derived cultures. The mutational landscape of individual patients varied widely, reflecting the heterogeneity of NSCLC and underscoring the need for individualized therapeutic approaches.
The value of this approach is demonstrated in patients TR33 and TR106. In patient TR33, a smoker, whole-exome sequencing revealed a high number of somatic SNVs. Mutational signature analysis revealed a dominant presence of the COSMIC signature SBS4, which is associated with tobacco exposure, while no clinically actionable point mutations were detected. However, copy number analysis revealed amplification of the HER2 gene. Despite the absence of actionable mutations, in ex vivo assays this patient's cells responded selectively and robustly to the EGFR inhibitor erlotinib, while remaining largely resistant to standard chemotherapeutics. In contrast, patient TR106 had a lower total number of somatic SNVs but had amplification of the PIK3CA gene and a PTEN missense mutation accompanied by loss of heterozygosity. Mutational signature analysis revealed the presence of SBS2 and SBS13, which are associated with APOBEC-mediated mutagenesis. Ex vivo drug testing showed broad sensitivity to almost all chemotherapeutic agents tested. The molecular profile of this patient, characterized by activation of the PI3K pathway and high APOBEC activity, may contribute to increased susceptibility to cytotoxic agents.

Conclusion: Our results show that the integration of whole-exome sequencing with functional drug profiling could be a powerful and clinically relevant strategy for personalized diagnostics in NSCLC. This combined approach enables comprehensive tumor characterization, reveals therapeutic vulnerabilities beyond known driver mutations and identifies exceptional responders. Such a strategy has the potential to improve patient stratification and make more effective, individualized treatment decisions in clinical practice.

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L24

CAR T cell therapy: A global perspective with a focus on Hungary's journey

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Keywords: CAR T cells, CAR NK cells, HER2, trastuzumab resistant tumors, gene modified immune cells

Chimeric Antigen Receptor (CAR) cell therapy represents a major milestone in the evolution of synthetic immunology, offering new treatment paradigms for cancer. While clinical success has been most striking in hematological malignancies, the application of CAR T cells to solid tumors such as breast cancer remains limited due to antigen heterogeneity, physical barriers to infiltration, and immunosuppressive microenvironments. At the Department of Biophysics and Cell Biology at the University of Debrecen, we have dedicated nearly two decades to developing innovative CAR-based approaches, particularly focusing on HER2-positive tumors and mechanisms of resistance to conventional monoclonal antibody therapies.

HER2 (human epidermal growth factor receptor 2) is a validated therapeutic target in breast cancer. Trastuzumab, a monoclonal antibody against HER2, has significantly improved outcomes for patients with HER2-overexpressing tumors. However, intrinsic or acquired resistance to trastuzumab remains a major clinical challenge, often associated with limited antibody penetration into tumor tissue or altered HER2 signaling. To overcome this, we developed HER2-

specific CAR T cells using trastuzumab-derived single-chain variable fragments (scFvs), enabling T cells to mimic and extend the targeting capabilities of the antibody. These engineered T cells exhibit cytotoxicity against HER2-positive tumor cells, even in settings where trastuzumab is ineffective. Crucially, our studies demonstrate that CAR T cells can penetrate deep into tumor spheroids and eradicate cells in areas poorly accessible to antibodies, offering a potential solution to the spatial limitations of passive immunotherapy.

Another critical factor influencing the success of CAR T cell therapy in solid tumors is the composition and functionality of the infused cell product. Our preclinical work has highlighted the essential role of $CD4^+$ T cells in CAR-mediated tumor eradication. These helper cells contribute not only to the orchestration of immune responses but also possess direct cytolytic potential when engineered with HER2-specific CARs. The maintenance of a $CD4^+$ population within the CAR T cell product was crucial for sustained antitumor activity, improved in vivo persistence, and reduced exhaustion in solid tumor models. This finding supports a strategy that deliberately preserves and expands $CD4^+$ T cells during CAR T cell manufacturing to optimize therapeutic outcomes.

The intracellular signaling domain of the CAR construct is another determinant of T cell behavior, impacting activation kinetics, persistence, and memory formation. We have conducted comparative analyses of CARs incorporating either CD28 or 4-1BB (CD137) costimulatory domains, as well as combinations of both. CD28-based CARs typically induce rapid effector responses but can lead to accelerated exhaustion, while 4-1BB domains support longer-term persistence and mitochondrial fitness. Interestingly, we observed that the choice of costimulatory domain not only alters intracellular signaling cascades but also affects the spatial organization and mobility of CARs on the T cell surface. These biophysical differences influence how CAR T cells engage with tumor cells and may contribute to variations in killing efficiency and durability. These findings provide a rationale for designing hybrid signaling motifs or context-specific CARs based on tumor type and therapeutic goal.

In addition to engineering individual T cells, we have explored modular CAR T platforms that can flexibly engage different tumor targets. One such innovation is a universal CAR system that uses a biotin-trastuzumab fusion molecule as an adaptor between the CAR T cell and the tumor antigen. This system allows for tunable control over antigen recognition, enabling the same CAR T cells to be redirected toward various biotinylated antibodies targeting different tumors. Using this strategy, we demonstrated effective infiltration and destruction of large HER2-positive tumor xenografts that were otherwise resistant to direct antibody therapies. The modularity of this approach offers significant advantages for treating heterogeneous tumors or for switching targets in response to antigen loss variants.

Parallel to our T cell–based work, we have also investigated the use of natural killer (NK) cells as alternative effector cells for HER2-targeted therapies. NK cells possess inherent cytotoxic potential and can be used in allogeneic settings without risk of graft-versus-host disease, making them attractive candidates for off-the-shelf immunotherapy. We engineered HER2-specific CAR NK cells using the NK-92 cell line and evaluated their efficacy against breast cancer cells. Our studies revealed a novel resistance mechanism: the expression of CD44, a surface glycoprotein linked to cancer stemness and epithelial-to-mesenchymal transition, significantly modulates the cytolytic capacity of HER2-CAR NK cells. Tumor cells with high CD44 expression exhibited reduced susceptibility to NK cell–mediated killing, highlighting the need to consider CD44 as a functional biomarker when designing CAR NK therapies. These results suggest that CD44 co-targeting or tumor cell reprogramming may enhance the effectiveness of HER2-CAR NK cell–based strategies.

Taken together, our findings underscore the importance of comprehensive CAR design that considers not only antigen specificity, but also effector cell type, costimulatory signaling, and modular targeting. The ability of CAR T cells to penetrate and eliminate trastuzumab-resistant tumor regions, the critical contribution of $CD4^+$ cells to antitumor responses, and the interplay between costimulatory domains and receptor dynamics all represent key variables that can be manipulated for improved outcomes. Meanwhile, CAR NK cells offer a complementary avenue for HER2 targeting, particularly in settings where T cell–based approaches are impractical or limited by toxicity.

As CAR-based therapies expand beyond hematologic cancers, a deeper understanding of their molecular, cellular, and physical interactions within the tumor environment will be essential. Our work contributes to this evolving field by offering mechanistic insights and preclinical validation of HER2-targeted strategies that overcome known resistance mechanisms. We aim to advance the next generation of adoptive immune cell therapies for solid tumors through sustained innovation and translational research, improving their efficacy, safety, and accessibility.

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L25

Targeting Multidrug Resistance and Cancer Stem Cells with Ionophores

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Keywords: Cancer stem cells, epithelial-to-mesenchymal transition, Golgi apparatus, Ionophores, multidrug resistance, salinomycin

Background: Cancer stem cell (CSC) populations are considered among the most therapy-resistant tumor cell populations and are believed to be key drivers of cancer relapse. Their resistance to chemo- and radiotherapy arises from several mechanisms. One such mechanism is epithelial-to-mesenchymal transition (EMT), a cell reprogramming process during which epithelial cells lose their polarity and adhesion properties and acquire mesenchymal traits, including motility. Epithelial carcinoma cells that have undergone EMT display CSC-like characteristics—such as invasiveness, drug resistance, and the ability to initiate metastases—thereby contributing to cancer progression and relapse [1]. Targeting the EMT program to eliminate CSCs selectively is thus a promising strategy to enhance cancer treatment efficacy.

It has been demonstrated that induction of EMT in immortalized human mammary epithelial cells (HMLEs) leads to the expression of stem cell markers and acquisition of the breast CSC phenotype. As such, HMLEs represent a robust experimental model of CSCs. High-throughput screening using this model identified several compounds with selective activity toward EMT/CSCs. The most potent was salinomycin (Sal), a naturally occurring K^+/H^+ ionophore widely used as a coccidiostat, followed by nigericin (Nig), another ionophore with similar selectivity [2]. Salinomycin has since been shown to be highly effective and selective against CSCs across multiple cancer types [3]. Despite numerous proposed mechanisms of action, the basis of its selectivity remains incompletely understood.

Ionophores are characterized by their ability to transport ions across lipid membranes, thereby disturbing membrane potential and ion homeostasis, which leads to physiological and osmotic stress. Consequently, natural ionophores (e.g., Sal, Nig, valinomycin) have been used as antibiotics and are being increasingly investigated in cancer treatment [4].

The EMT/CSC phenotype is often associated with overexpression of ATP-binding cassette (ABC) transporters, including ABCG2 and P-glycoprotein (P-gp), which contribute to multidrug resistance (MDR) by preventing the intracellular accumulation of chemotherapeutic agents. Therefore, modulation of ABC transporter activity or expression is of great clinical importance for overcoming MDR and improving the efficacy of anticancer therapies [5] Previous studies have shown that some well-known K^+/H^+ ionophores inhibit P-gp and sensitize resistant leukemia cells to chemotherapeutics such as adriamycin, docetaxel, and vinblastine [6]. However, their effects on ABCG2 activity have not been systematically addressed [4].

Our group has extensively studied the biological and potential antitumor activity of various compounds, particularly focusing on CSC selectivity. These include ion transport-disrupting agents such as crown ether compounds and natural ionophores (e.g., Sal, monensin (Mon), and Nig). In our recent work, we investigated the mechanisms underlying the CSC/EMT-selective activity of ionophores.

Materials and Methods: Human myeloid leukemia cell line PLB-985/ABCG2 and Madin-Darby canine kidney cells type II (MDCKII/ABCG2), both overexpressing human ABCG2, as well as their parental counterparts (PLB-985 and MDCKII, respectively), were used as model systems. A range of functional assays was employed to study the interaction of the ionophores salinomycin, nigericin, and monensin with the ABCG2 transporter. Inhibitory activity on ABCG2 function was assessed using the Pheophorbide A (PhA) efflux assay or the Hoechst 33342 accumulation assay in MDCKII/ABCG2 and PLB-985/ABCG2 cell lines.[7]

Additionally, a mammary CSC model generated by EMT induction in immortalized human mammary epithelial (HMLE) cells was used. These included HMLE cells expressing Twist or an empty vector (HMLE-Twist and HMLE-pBp), as well as HMLE cells with shRNA targeting E-cadherin or GFP (HMLE-shEcad and HMLE-shGFP). Other breast cancer cell lines, such as MCF-7 and SUM159, were also included [2],[8].

Cell proliferation was assessed using the MTT assay and flow cytometry. Protein expression was evaluated by Western blotting and flow cytometry. Cell morphology and specific protein localization were analyzed using confocal microscopy. Global gene expression profiling (RNA-Seq) was performed using the NextSeq500 platform (Illumina). Sequence reads were uploaded to the BaseSpace Sequence Hub (Illumina) and aligned to the human reference genome (hg19) using the RNA-Seq Alignment app. Differential gene expression after treatment, for each cell line and between EMT and non-EMT cells, was analyzed using the DESeq2 app. The N-glycome of secreted proteins was analyzed by mass spectrometry.

Results: We found that Sal, Mon, and Nig do not inhibit ABCG2 activity, suggesting that their CSC selectivity arises

through alternative mechanisms [9],[10]. Notably, we discovered that Sal disrupts the function of the Golgi apparatus, similarly to Mon, a known Golgi disruptor. This prompted further investigations to clarify whether salinomycin's selectivity is linked to its ability to impair Golgi function.

Effect of ionophores on ABCG2 activity.

Several studies have reported that natural ionophores (e.g., Sal, Mon, and Nig) sensitize resistant cancer cells to various chemotherapeutics, an effect primarily attributed to their inhibitory activity on ABCB1 (P-gp). In contrast to this well-documented P-gp-mediated multidrug resistance (MDR) reversal, our current study did not demonstrate any inhibitory activity of these ionophores on ABCG2. Moreover, salinomycin may act as a substrate of ABCG2 rather than an inhibitor. This implies that their CSC-selectivity is likely mediated by distinct, transporter-independent mechanisms [7].

Influence of salinomycin on Golgi function in EMT cells.

We further confirmed that Sal reduced the proportion of EMT cells in a mixed population. In proliferation assays, Sal showed a more pronounced inhibitory effect on EMT cells compared to non-EMT cells. However, this selectivity diminished at higher concentrations of Sal, which were cytotoxic to both cell types. These higher doses also caused immediate mitochondrial depolarization, which was even more prominent in non-EMT HMLE-pBp cells. This suggests selective toxicity toward EMT cells, albeit to a lesser extent than previously reported.

Notably, we demonstrated that Sal disrupts Golgi function, similar to Mon. Both compounds altered GA morphology, but only EMT cells exhibited extensive fragmentation of the Golgi and ER-Golgi intermediate compartments, and were sensitized to Sal and Mon treatment. Furthermore, Sal induced the expression of ER-Golgi-related genes predominantly in EMT cells. Impaired Golgi function led to alterations in post-translational modifications—specifically, reduced protein secretion and significant changes in the N-glycosylation profile of secreted proteins, most notably a reduction in complex N-glycans.[8] Strikingly, cell adhesion emerged as the most affected biological process in EMT cells treated with Sal, which was not the case in non-EMT cells.

Conclusions: Our data clearly establish salinomycin (Sal) as a Golgi-disrupting agent and support existing evidence that cells undergoing EMT are particularly sensitive to Golgi perturbation. These findings further elucidate the mechanism underlying the selective activity of Sal and related ionophores toward EMT/CSC cells and underscore the therapeutic potential of Golgi-disrupting compounds in targeting cancer stem-like populations. Moreover, they point to a broader and previously underappreciated role of the Golgi apparatus in regulating EMT plasticity.

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L26

Dual targeting concept: hybrid molecules of cisplatin and antiinflammatory drugs

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Keywords: antiinflammatory drugs, chemotherapy, drug delivery, platinum

Background: Recently, it has become evident that dying cells play a central role in promoting cell division and serve as a primary source of signals driving tumor repopulation and the development of an aggressive phenotype. Prostaglandin E2 (PGE2), a proinflammatory molecule, has a prominent role in delivering a mitogenic signal to neighboring cells, thus providing a therapeutic opportunity against cancer. With an aim to block mitogenic signal of PGE2, we created hybrid molecules composed of nonsteroidal antiinflammatory drugs and conventional platinum-based cytostatic. This multitargeting approach enables induction of cell death without tumor repopulation feedback. In addition to official cyclooxygenase 2 (COX-2) inhibitors, many unexplored plant metabolites, for instance alkyl esters of caffeic acid (CA), demonstrate significant enzyme inhibition. To further improve antitumor potential of novel compounds, mesoporous nanosilica was employed as drug delivery vehicle. In this study Pt(IV) complexes based on cisplatin scaffold bearing naproxen, ibuprofen, flurbiprofen, and derivative of CA, loaded into silica SBA-15 were evaluated in 2D and 3D cell culture as well as in vivo syngeneic mouse model of melanoma, breast and colon cancer.
Material and Methods: The cytotoxicity of four conjugates on 4T1, B16, CT26, and MC38 cell lines was estimated by MTT and CV assays. Organoids were established from MC38-induced tumors in C57BL/6 mice which were then isolated, digested and further propagated. CellTiter-Glo™ luminescent assay was employed for evaluation of the drugs' effect on organoids. In vivo efficacy was investigated in tumor-challenged C57BL/6 and BALB/c mice. Flow cytometry and microscopy were employed in order to enlight the mechanism of action of selected conjugates.
Results: All tested hybrid drugs significantly reduced viability of cell lines in 2D cultures with high reproducibility in 3D cultures. In mouse model of melanoma, breast and colon cancer, tumor shrinkage and a better toxicity profile comparing to cisplatin were noticeable. Mechanistic insight on melanoma in vitro has indicated a pro-senescent action of selected drugs.
Conclusions: This strategy of combining cytotoxic drug and antiinflammatory agents holds great potential for developing treatment options that simultaneously target cancer cells and intercellular network in tumor microenvironment.

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L27

Acrylamide in Food and Its Carcinogenic Potential: What Do We Really Know?

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Keywords: acrylamide, carcinogen, food

Acrylamide, a chemical compound formed in carbohydrate-rich foods during high-temperature cooking processes such as frying, baking, and roasting, has been classified as a probable human carcinogen based on animal studies. These studies have demonstrated that acrylamide and its metabolite, glycidamide, can cause DNA damage and increase cancer risk in rodents. Acrylamide has been demonstrated to exert deleterious effects in experimental animals. Evidence has emerged that this substance is toxic to certain tissues, including reproductive tissues and the retina. In addition, studies have indicated that it is neurotoxic to rats and mice. The substance undergoes conversion to glycidamide, a highly reactive epoxide, by CYP2E in humans. This process results in the acquisition of genotoxic and carcinogenic properties

by the substance, which then affects multiple organs. A number of epidemiological studies have been conducted in an attempt to ascertain whether there is an association between occupational and dietary exposure to acrylamide and the incidence of cancer. However, the results of these studies have not indicated any such association. A number of studies have demonstrated a correlation between acrylamide intake and the occurrence of cancers in renal cells, the endometrium, and the ovary. In conclusion, while acrylamide has demonstrated carcinogenic potential in animal models, current human studies provide limited and inconclusive evidence regarding its role in cancer development. Further research, particularly studies utilizing biomarkers of exposure and accounting for genetic and lifestyle factors, is necessary to clarify the potential health risks of dietary acrylamide.

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L28

The role of microbiota in urinary bladder cancer

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Keywords: microbiota, carcinogens, bladder neoplasms

Exposure to environmental pollutants and the composition of the human microbiome are increasingly recognized as significant factors predisposing individuals to tumor development. While the biotransformation of xenobiotics, including pollutants, by human-associated microorganisms has been shown to influence toxicokinetics and tissue exposure to these compounds, the impact of such microbial activity on chemical-induced carcinogenesis remains inadequately understood. Our research demonstrates that depletion of the gut microbiota significantly alters the toxicokinetics of nitrosamines, resulting in reduced incidence and severity of nitrosamine-induced urinary bladder cancer in mice. By employing both in vitro and in vivo methodologies, including individualized human gut bacterial cultures and gnotobiotic mouse models, we causally linked gut bacterial biotransformation of nitrosamines to carcinogen metabolism. Importantly, we found that this metabolic activity varies among individuals according to their specific microbial profiles and is relevant across structurally related nitrosamine carcinogens. These findings indicate that the gut microbiota's metabolic capacity may be a critical, yet previously underappreciated, modifier of risk for chemical-induced cancer, offering new possibilities for microbiome-targeted risk assessment and preventive strategies. Building on this foundation, we investigated the urinary microbiome in bladder cancer by analyzing urine samples from bladder cancer patients and healthy controls using 16S rRNA sequencing. Firmicutes were the most abundant phylum across groups, and while overall composition and diversity did not differ significantly, specific operational taxonomic units (OTUs) varied: Fusobacterium nucleatum, a genus linked to protumorigenic activity, was enriched in cancer patients. In contrast, OTUs corresponding to Veillonella, Streptococcus, and Corynebacterium were more prevalent among healthy controls. Lastly, we examined the dynamics of urinary microbiota during BCG immunotherapy for high-risk non-muscle-invasive bladder cancer. Microbiome composition changed throughout treatment, and differences between responders and non-responders were observed pre-therapy, specifically, higher Aureispira and lower Negativicoccus succinivorans in non-responders.
Collectively, these findings highlight the active role of urinary microbiota in bladder cancer pathogenesis and treatment and underscore the need for further research into microbiome-based biomarkers and intervention strategies.

L29

Tumor metabolism in the picture: Tracing and visualizing the metabolic alterations in cancer

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Keywords: cancer, metabolism, mass spectrometry imaging, spatial metabolomics, metabolic flux

Metabolic reprogramming is a hallmark of cancer, enabling tumor cells to meet the demands of rapid proliferation, survival in hostile microenvironments, and therapeutic resistance. This lecture will explore how advanced metabolomics approaches are employed to dissect these metabolic alterations in cancer. Steady-state metabolomics allows for the quantitative profiling of a snapshot of intracellular metabolites, revealing pathway enrichment and dysregulations. Stable-isotope tracing provides dynamic insights into carbon and nitrogen fluxes through metabolic networks, identifying nutrient preferences and pathway plasticity in cancer cells. Meanwhile, spatial metabolomics, by mass spectrometry imaging (MSI), offers a visualization of the metabolic heterogeneity spatially resolved within tumors and the tumor microenvironment. By integrating these methodologies, researchers can not only quantify metabolic fluxes and pathway dependencies but also visualize spatial metabolic heterogeneity within tumors and systematically characterize the metabolic dependencies of cancer cells to uncover context-specific vulnerabilities. In this session, selected research studies will be discussed to illustrate how these tools are used in experimental designs to study tumor progression, therapeutic responses and metabolic adaptation, providing a functional framework for targeting cancer metabolism.

L30

Changes in tumor metabolism, consequences, and therapeutic opportunities

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Keywords: acidosis, alkalization treatment, cancer metabolism, sodium bicarbonate, tumor microenvironment

Cancer cells undergo profound metabolic reprogramming to sustain their rapid proliferation and adapt to stressful microenvironmental conditions. In addition to the well-known Warburg effect—where tumor cells preferentially utilize aerobic glycolysis over oxidative phosphorylation—even more metabolic alterations occur, such as enhanced glutaminolysis, dysregulated lipid metabolism, altered one-carbon metabolism, and modified mitochondrial function.
A major consequence of this metabolic shift is the acidification of the tumor microenvironment. High rates of glycolysis lead to excessive lactate production and proton accumulation, resulting in an extracellular pH typically ranging from 6.5 to 6.9—considerably lower than the neutral pH (~7.4) of normal tissues. This acidic environment fosters tumor progression by enhancing invasion and metastasis through the activation of matrix metalloproteinases, which degrade the extracellular matrix. Moreover, acidosis impairs immune surveillance by suppressing the function of cytotoxic T cells and natural killer cells, allowing cancer cells to evade immune detection and destruction. Tumor acidity also contributes significantly to therapeutic resistance. Acidic conditions reduce the efficacy of chemotherapy and radiation therapy, partly by activating survival pathways such as HIF-1α, NF-κB, and autophagy.
Given these challenges, targeting tumor acidity has emerged as a promising therapeutic strategy. One approach involves using sodium bicarbonate ($NaHCO_3$), a simple alkalizing agent capable of selectively neutralizing extracellular acid without altering systemic pH. Preclinical studies have shown that oral $NaHCO_3$ can raise tumor extracellular pH, inhibit metastasis, and enhance the effectiveness of chemotherapy. In addition, localized delivery methods such as intraperitoneal perfusion of $NaHCO_3$ have been explored, particularly in preclinical models of peritoneal carcinomatosis. This approach improves drug penetration into tumor nodules, and enhances chemotherapeutic efficacy, with minimal risk of systemic alkalosis.
Clinical evidence remains limited but encouraging. Pilot trials suggest that bicarbonate supplementation is safe and may delay disease progression or stabilize advanced cancers when used as an adjuvant. Although larger clinical trials

are needed, these findings support the potential role of alkalization strategies in oncology.

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L31

Interplay between redox regulation and cancer metabolism

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Keywords: cancer, metabolism, antioxidant, NRF2, aquaporin

Background: Redox regulation is vital for maintaining normal cellular function. Key to this regulation is the control of the flux of hydrogen peroxide (H_2O_2), which can be a key event, as H_2O_2 functions as a signaling molecule at low concentrations but is cytotoxic at high levels. Pores that mediate H_2O_2 permeability across membranes are therefore critical in maintaining redox homeostasis. Therefore, aquaporins (AQPs), initially defined as water channels, have emerged as important players. Aquaglyceroporins, a group of aquaporins, facilitate not only water but also small solute transport, including H_2O_2 and glycerol. By modulating H_2O_2 permeability, AQPs can influence redox-sensitive signalling pathways involved in tumor development and progression. Moreover, by facilitating glycerol transport, AQPs can regulate metabolic pathways, suggesting an important role in cancer cell physiology.
Materials and Methods: This study focuses on aquaglyceroporin AQP3 in breast cancer cell lines. Our primary objective was to investigate the role of AQP3 in mediating glycerol flux across the plasma membrane and its impact on cellular metabolism. We also assessed how glucose levels influence AQP expression. Furthermore, we explored whether NRF2, the master regulator of antioxidant responses, can modulate the expression of AQP3 and AQP5.
Results: Our results demonstrate that glucose levels influence the expression of AQP as well as NRF2. Also, modulation of NRF2 influences the levels of AQP3.
Conclusions: Our results suggest a potential regulation between NRF2 and AQPs, suggesting that redox and metabolic pathways are closely linked via these membrane channels. The observed glucose-dependent modulation of both AQPs and NRF2 indicates possible crosstalk that could contribute to the metabolic flexibility and stress resistance of cancer cells.

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L32

Tracking Osteosarcoma Progression: A Longitudinal Single-Patient Study Integrating Functional Analyses and Single-Cell Transcriptomics

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Keywords: cancer stem cells, chemotherapy resistance, telomere extension, osteosarcoma, single-cell RNA sequencing

Background: Osteosarcoma (OS) is the most common malignant bone tumor, peaking in adolescence and later in adulthood. Despite defined histological subtypes and grading, predicting disease progression remains challenging due to its rarity, genetic complexity, and heterogeneity. While most cases arise sporadically, some are linked to inherited syndromes such as Li-Fraumeni syndrome or retinoblastoma. Advances in chemotherapy have improved survival for localized OS to over 65%, yet outcomes for metastatic disease remain poor. Tumor progression and recurrence are driven by accumulating mutations and selective pressures[1] , leading to therapy-resistant, highly adaptable cell populations. A deeper molecular understanding is crucial for improving prognostic and therapeutic strategies.
To better understand these dynamics, we conducted a longitudinal study tracking tumor evolution in a single patient across three key clinical stages: diagnosis, after neoadjuvant chemotherapy, and recurrence. The patient, a young male diagnosed with grade 3 chondroblastic OS of the femur, was treated with the EURAMOS-1 chemotherapy protocol [2] followed by surgical resection and additional adjuvant therapy. Upon recurrence, a second surgery was performed. Tumor samples were collected at each stage, and primary cell cultures were established and enriched for cancer stem cells (CSCs). These cultures were then evaluated using a multimodal platform that included morphological and biophysical analyses, functional assays, and single-cell RNA sequencing (scRNA-seq).

Materials and Methods:

Patient Samples and Ethical Approval

Tumor material was obtained from a male patient with grade 3 chondroblastic osteosarcoma of the femur at three time points: diagnostic biopsy, post-neoadjuvant chemotherapy resection, and resection following recurrence. All samples were collected at the Children's Hospital in Zagreb with ethics committee approval and informed consent. U2OS (osteosarcoma) and HeLa (cervical carcinoma) cell lines were used as experimental controls.

Primary Cell Culture and CSC Enrichment

Primary cell cultures were established from all tumor samples. Cancer stem cells (CSCs) were enriched using spheroid culture in serum-free DMEM/F-12 medium supplemented with FGF2, EGF, ITS, ammonium bicarbonate, progesterone, putrescine, and 2% methylcellulose on low-attachment plates. Adherent 2D cultures were maintained in DMEM/F-12 with 10% fetal bovine serum (FBS) and antibiotics. Cells were passaged at 80–90% confluency and used between passages 3 and 7.

Spheroid Formation Assay and Imaging

To quantify CSC frequency, monolayer cells were dissociated into single-cell suspensions and seeded into low-attachment 96-well plates at a density of 10 cells/µL. Spheroids were cultured for seven days and analyzed by inverted microscopy. Spheroid-forming efficiency (SFE) was calculated as:
SFE (%) = (number of spheroids formed / number of seeded cells) × 100.
Adherent cell morphology was evaluated using the HoloMonitor® Live Cell Imaging System over 48 hours. Monitored parameters included optical thickness, shape irregularity, and perimeter.
Spheroid properties (mass density, diameter, and weight) were assessed with the W8 device (CellDynamics) based on sedimentation analysis [3]. Spheroids were grown for 10 days, fixed in 4% PFA, and analyzed in duplicate. Statistical evaluation was performed using Student's t-test.
3D hydrogel-based spheroid imaging was performed using tyramine-conjugated gelatin and hyaluronic acid hydrogels, crosslinked with HRP and H_2O_2. Spheroids were imaged every 30 minutes for seven days. Differences between conditions were analyzed using the Kruskal–Wallis test followed by the Bonferroni-corrected Dunn's post hoc test.

Migration Assay

To assess migratory potential, a wound healing assay was performed. Once cell confluence was reached in 6-well plates, a scratch was made, and cell migration was monitored every 3 hours for 48 hours using HoloMonitor imaging. Cells were maintained in serum-free medium to suppress proliferation. Migration was quantified using dedicated software.

Telomere Length and hTERT Expression

Relative telomere length was measured using qPCR based on the Cawthon method. Telomere primers and IFNB1 were used to calculate the T/S ratio. hTERT expression was quantified in patient-derived and control cell lines by RT-qPCR, normalized to β-actin, and expressed relative to HeLa cells. Data were analyzed using one-way ANOVA with Tukey's post hoc test.

Chemosensitivity Testing

Drug resistance was assessed using MTT (2D cultures) and CellTiter-Glo 3D assays. Cells were treated with cisplatin, doxorubicin (0–100 µM), or methotrexate (0–1500 µM) and viability was measured after 72 hours. In 3D conditions, spheroids were formed before treatment. All treatments were performed in triplicates with appropriate controls.

Single-Cell RNA Sequencing and Analysis

Primary patient-derived cells (passage 3) were prepared for scRNA-seq using the 10x Genomics Chromium platform. Libraries were generated with the Next GEM 3' v3.1 kit and sequenced on an Illumina NovaSeq. Raw data were processed using Cell Ranger and Seurat. Quality filtering excluded cells with <350 genes or >10% mitochondrial reads. Doublets and low-expression genes were removed.

Dimensionality reduction and batch correction were performed using PCA, CCA, RPCA, and Harmony integration. Clusters were identified using the Louvain algorithm (resolution 0.4) and visualized via t-SNE. Annotation was guided by Azimuth and Human Primary Cell Atlas references. Differentially expressed genes (DEGs) were defined using Wilcoxon rank-sum tests with the Bonferroni correction.

Gene Enrichment and Pathway Analysis

DEGs were used for over-representation analysis (ORA) of Gene Ontology (GO) and Hallmark pathways. Redundant GO terms were filtered using the simplify function. Gene set enrichment analysis (GSEA) was performed with the singleseqgset package. DEGs between stages within specific clusters were identified using the FindMarkers function (FDR < 0.05 and ≥30% detection difference).

Results: Single-cell RNA sequencing of osteosarcoma cells collected at diagnosis, after chemotherapy, and at recurrence revealed six transcriptionally distinct clusters with dynamic shifts in abundance over time. Clusters 1–3 were enriched in proliferative and cell cycle genes (PLK1, AURKA, HIST1H1), with a reduction in cluster 1 at recurrence. Cluster 4, predominant post-chemotherapy, expressed tumor suppressors (CITED2, PNRC1) and stress-related genes, suggesting a transient quiescent state.

Clusters 5 and 6 were most prominent at recurrence. Cluster 5 showed a chondroblastic signature, including ACAN and anti-apoptotic MTRNR2L12. Cluster 6 exhibited features of cancer stem cells (CSCs), expressing NES, ALDH1A1, and metastasis-associated genes such as NCAM1 and ANKRD1. Additionally, it overexpressed myogenic markers (MYOD1, DES, PAX7), suggesting a muscle-like migration mode distinct from classical EMT [4].

Functional assays supported these transcriptomic findings. CSC frequency increased from diagnosis to recurrence (~0.0036%), and recurrence-stage cells demonstrated enhanced motility in wound healing assays. Live-cell imaging revealed morphological changes, with recurrence cells showing greater perimeter, irregular shape, and reduced optical thickness—consistent with increased invasiveness. Recurrent-stage spheroids were larger and less dense, indicating reduced cohesion and greater structural plasticity.

Telomere maintenance also shifted during disease progression. Although telomeres shortened and hTERT expression was undetectable, proliferative capacity was preserved via alternative lengthening of telomeres (ALT). Cluster 6 showed elevated BLM expression, a key enzyme in ALT [5], suggesting this mechanism supports immortality in aggressive cell populations.

Chemosensitivity varied by stage and culture model. Doxorubicin sensitivity increased overall, though ALDH1A1 expression in cluster 6 may contribute to resistance through detoxification. Cisplatin resistance was highest in 3D cultures at recurrence, linked to low drug uptake (SLC31A1) and upregulation of DNA repair genes and the apoptosis inhibitor XIAP. Methotrexate resistance persisted and was associated with overexpression of DHFR and ADA, despite low transporter expression.

These results identify cluster 6 as a mesenchymal, therapy-resistant CSC population with muscle-like migratory features and broad resistance mechanisms. The study's longitudinal design—tracking tumor evolution within a single patient—enabled direct observation of how subclones adapt and survive therapy. Despite limitations from in vitro culturing, this approach allowed functional validation of transcriptomic findings. Cluster 6 emerges as a promising therapeutic target marked by ALT activity, EMT/myogenic programs, and chemoresistance.

Conclusion: Unlike most OS studies, which are cross-sectional and compare unrelated patient samples [6], our longitudinal, within-patient approach eliminates interpatient variability and enables direct observation of clonal shifts and therapy-driven selection. Although this model lacks the native tumor microenvironment, it enabled functional testing and the enrichment of CSC-like populations. This study highlights the emergence of a highly plastic, drug-resistant CSC-like population during osteosarcoma progression. Functional and transcriptomic profiling identified cluster 6 as a key driver of recurrence, featuring ALT-mediated immortality, myogenic remodeling, and resistance to

multiple therapies. These findings underscore the need for advanced 3D models and support targeting ALT, XIAP, DHFR, and cytoskeletal regulators in future treatment strategies.

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L33

Bridging the Gap: The Role of PDTX Models in Cancer Therapy Development

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Keywords: animal experimentation, disease models animal, drug development, drug evaluation

Background: The continuous need for more effective anticancer therapies has driven the production of a vast number of drug candidates. These compounds are typically subjected to rigorous preclinical testing, often relying on traditional rodent xenograft models. While these models provide useful pharmacokinetic data, their reliance on cell line-derived xenografts (CDX) poses significant limitations. CDX models lack the cellular and genetic diversity as well as the spatial characteristics of patient tumors, leading to discrepancies between preclinical outcomes and clinical efficacy [1]. As a result, many promising drug candidates fail in clinical trials, with a reported attrition rate of approximately 95%. The majority of these failures stem from a lack of efficacy which is issue that could potentially be identified earlier with more predictive models. In this context, Patient-Derived Tumor Xenograft (PDTX) models offer a transformative approach to cancer drug development.PDTX models are established by directly implanting patient tumor tissue into immunodeficient mice, thereby preserving the original tumor's architecture, heterogeneity, and microenvironment [2]. Unlike CDX models or conventional 2D cultures, PDTXs better mimic human tumor behavior and response to therapy, providing a clinically more relevant platform for drug screening.With the field of oncology increasingly oriented towards precision medicine, the role of PDTX models is gaining prominence. They help reduce clinical trial failures, streamline drug development timelines, and lower associated costs. By bridging the translational gap between preclinic and clinic studies, PDTX models represent a vital step towards more effective and individualized cancer treatments. Patient-Derived Tumor Xenograft (PDTX) models have become a cornerstone in translational oncology, accurately modeling human cancers and more realistic drug response patterns. Unlike conventional cell line-based models, PDTXs preserve the essential biological features of the original clinical tumors, making them uniquely suited for preclinical drug development and personalized therapy approaches.PDTX models preserve key properties of clinical

tumors, maintaining close molecular, morphological, and immunohistochemical similarity to human cancers [3]. They faithfully reproduce the microenvironment and cellular heterogeneity found in clinical settings, allowing the tumor structure and behavior to remain stable across several generations of host animals. Moreover, PDTX models mirror tumor metabolism and vascularization, accurately reflecting nutrient flow and drug distribution as it occurs in patients. Importantly, these models retain functional activity: the tumor cells remain viable and biologically active through serial transplantation, enabling long-term study of tumor dynamics and drug interactions.In addition to preserving tumor integrity, PDTX models provide valuable advantages in the development of cancer therapies. These models offer a more dependable platform for in vivo drug testing, increasing the likelihood that preclinical findings align with clinical outcomes. Critically, PDTX-derived organoids (PDTOs) [4] enhance the predictability of clinical therapeutic response in a quick, cost-effective way, contributing to enhanced decision-making in personalized medicine. PDTX models represent a transformative advance in preclinical oncology, offering a more accurate and clinically predictive framework for the development and evaluation of cancer therapies, enabling a shift from simplified cell-based assays to biologically complex, patient-mimicking systems [5]. Their application in drug efficacy testing, biomarker validation, resistance mechanism studies, and co-clinical trial design is crucial in reliable cancer therapy development. Vascularization and tumor stroma are preserved, making PDTX a vital platform to test modalities that do not directly target cancer cells but instead kill tumors via tumor microenvironment (TME) alteration. Furthermore, they have application for in-detail genomic analysis of tumor stages: size-dependent, metastasis vs. primary tumor effects, drug naïve vs. treated vs. resistant, etc. On the other hand there are limitations to using PDTX models [3]. Antibodies and immunotherapies cannot be properly tested with this technology, while murine antibodies can be tested in allografts or Genetically engineered mouse models (GEMMs) or short-term in humanized mice. Lack of a human immune system: PDTXs are typically implanted into immunodeficient mice to avoid rejection, but this prevents accurate modeling of immune responses, making them unsuitable for studying immunotherapies. Engraftment bias: not all patient tumors successfully engraft, where fast-growing, aggressive tumors are more likely to take hold, potentially skewing research away from slower-growing but clinically relevant cancers. Loss of stromal components: human stromal and immune cells in the tumor microenvironment are gradually replaced by murine cells, which can alter tumor–host interactions and drug responses over time. Routine use of PDTX banking in clinical centers is urgent but complex, time-consuming, and costly, requiring cooperation of surgical, pathological, administrative (patient consent) and research teams/animal house/biobank.

Results: Our lab develops patient-derived tumor xenograft (PDTX) models that preserve tumor heterogeneity and microenvironmental complexity, offering a highly predictive platform for studying therapeutic responses. By sourcing samples from numerous cancer types, we have established a diverse and well-documented PDTX model collection, including rare malignancies and metastatic lesions. These models support both basic and translational research by mimicking clinically relevant tumor behavior in vivo.Our biobanked PDTX models are serially xenografted in immunodeficient mice and are accompanied by complete histological and molecular documentation. Additionally, we establish PDTX-derived cell line cultures (PDTC) or organoids (PDTO) to compare in vitro and in vivo tumor behavior, enhancing our understanding of model fidelity and tumor dynamics, and effectively prescreening and extensively study possible drug effects. As part of our research activity, within a wide range of PDTX models from different tissue origins and molecular signature, we have developed a vemurafenib-resistant PDTX model from a BRAF V600E-mutant melanoma patient [6]. This model, characterized both molecularly and morphologically, was subjected to long-term BRAF inhibitor vemurafenib treatment, resulting in an in vivo therapy-resistant phenotype. Bulk mRNA sequencing analysis did not align with previously described resistance mechanisms, prompting further investigation of novel, differentially expressed genes. Notably, in this model we also found the role of the ABCB1 multidrug transporter, a rarely studied factor in melanoma, which may contribute to drug efflux and therapeutic resistance, given vemurafenib is its substrate and can be expelled from ABCB1-positive cells. Furthermore, the same model was successfully used to identify potentially druggable metabolic changes affecting cysteine metabolism during the evolution of resistance to dabrafenib-trametinib dual treated melanoma [7]. PDTX platform enables the discovery of resistance-driving pathways and facilitates preclinical testing of next-generation therapies. By focusing on complex and underrepresented tumor models, we aim to accelerate the development of personalized cancer treatments and support metastasis-targeted drug discovery by collecting multiple samples and establishing multiple PDTXs from the same patient's primary and disseminated tumor sites.

Conclusion: PDTX models serve as a critical bridge between basic research and clinical application by preserving tumor heterogeneity, microenvironment, and drug response profiles. They offer a more accurate and predictive platform than traditional models, supporting drug development, resistance mechanism discovery, and personalized therapy design. Despite certain limitations, their integration into translational research enhances the ability to model real-world tumor behavior and identify more effective cancer treatments. PDTX models are reshaping the approach to precision oncology and accelerating the path from bench to bedside.

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L34

Epi-CRISPR tool: A synthetic epigenetic editing platform for cell reprogramming and therapeutic targeting in cancer

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Understanding and manipulating the epigenetic regulation of cell identity and disease progression holds immense therapeutic potential, particularly in light of recent advances in programmable epigenetic editors. The advent of CRISPR-based epigenetic systems, including dCas9-fused methyltransferases, histone modifiers, and chromatin remodelers, has expanded our ability to modify gene expression without altering the DNA sequence, offering novel routes for disease modeling and intervention.

Here, we present Epi-CRISPR, a next-generation synthetic epigenetic editing platform built on the latest developments in CRISPR/dCas9 technologies. Our system utilizes a controllable vector to precisely and reversibly edit epigenetic marks such as DNA methylation at targeted genomic loci. It has already been used to reprogram pancreatic α-cells into insulin-producing cells by modifying the epigenetic and transcriptional states of the ARX gene, a master regulator of pancreatic lineage specification. This work builds upon recent studies demonstrating that targeted reactivation or suppression of developmental genes can drive functional cell conversion, positioning Epi-CRISPR as a powerful tool for diabetes therapy.

Additionally, we explore Epi-CRISPR's application in inducing a BRCAness phenotype in triple-negative breast cancer (TNBC) cells lacking BRCA1 mutations. By directing locus-specific DNA methylation to the BRCA1 promoter, we suppress gene expression and investigate associated chromatin changes using high-resolution EPIC methylation. We further examine the interplay between DNA methylation and histone marks (H3K4me3, H3K9me3, H3K27me3) using ACT-seq methodology. This approach leverages recent findings on the dynamic cross-talk between DNA and histone modifications in cancer progression and therapy resistance.

By enabling programmable, non-mutagenic control over gene regulation, Epi-CRISPR holds promise as a clinically translatable strategy for enhancing cell plasticity, sensitizing tumors to targeted therapy (e.g., PARP inhibitors), and uncovering fundamental mechanisms of epigenetic memory and identity. Furthermore, epigenetic editing holds promise in overcoming drug resistance, modulating tumor immunogenicity, and personalizing treatment regimens based on epigenetic signatures. As epigenome-targeting tools become more refined and clinically compatible, epigenetic editing is poised to complement or even replace traditional therapies in selected cancer subtypes.

L35

Palladium (II) Based-Anticancer Compounds: A New Hope or Not?

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Cancer patients still need more effective chemotherapeutic/general cytotoxic drugs. Although target-based treatments have become more common in recent years, chemotherapy is still necessary. Moreover, the combination of cytotoxic and targeted therapy is expected to provide superior treatment options compared to chemotherapy alone. Synthesis of new palladium (II) (Pd(II)) complexes provides a new arsenal in this regard. Therefore, the designation and creation of new palladium (II)-based compounds are increasing. To date, many different compounds containing palladium (II) as a metal have been synthesized and tested in terms of their cytotoxic activities against various tumor types. It seems that the ligands attached to palladium (II) result in big differences in the cytotoxic potential of the complexes. In the literature, varying ligands have already been used, such as pyrazoles, oxamate, triphenylphosphine, 2, 6-diacetylpyridine bis (p-chlorophenylthiosemicarbazone, thioures, hydrazine, tetraaza [N 4], sulfur, thiosemicarbazones, hydrazine, oxalato, proflavine, triazole, organoarsenic, and curcumin. All these new Pd(II)-based compounds yield different cytotoxic potentials depending on the tumor types. Our group has broad experience with diverse metal complexes including copper-based ones. Taken together, in this talk, novel Pd(II)-based compounds synthesized by other groups will be discussed in comparison with our Pd(II)-based compounds [1-7]*in vitro* and *in vivo*.

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L36

From Tumor Suppression to Stress Response: The Diverse Roles of Sirtuin 3 in Cancer and Sex-Dependent DNA Damage Adaptation

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Keywords: Sirtuin 3, breast neoplasms, tumor suppressor proteins, DNA damage, sex factors

Background: Sirtuin 3 (Sirt3), a mitochondrial NAD^+-dependent deacetylase, regulates mitochondrial metabolism, oxidative stress, and cell survival. Its context-dependent role spans from functioning as a tumor suppression in estrogen-sensitive and triple-negative breast cancer (TNBC) to facilitating stress adaptation in non-transformed cells. However, the mechanisms through which Sirt3 modulates cell fate across various conditions remain incompletely understood.

Materials and Methods: To investigate the role of Sirt3 in cancer and stress response, we used MCF-7 (ER-α^+) and MDA-MB-231 (TNBC) breast cancer cells stably transfected with Sirt3 or control vectors. Assays included Western blotting, qPCR, flow cytometry, metabolic activity, mitochondrial function, and colony formation. Confocal microscopy and immunocytochemistry assessed protein localization. Separately, primary male and female mouse embryonic fibroblasts (MEFs) with or without Sirt3 were exposed to etoposide-induced DNA damage to evaluate sex-specific stress responses.
Results: in MCF-7 cells, Sirt3 overexpression reduced estrogen-induced proliferation, colony formation, and S-phase progression by stabilizing p53 and disrupting its interaction with ER-α. This was accompanied by increased oxidative phosphorylation and a metabolic shift unfavorable for tumor growth. In MDA-MB-231 cells, Sirt3 enhanced mitochondrial biogenesis, ROS, and metabolic activity, but also elevated DNA damage, apoptosis, and the formation of multinucleated cells. In MEFs, Sirt3 loss led to sex-specific responses to DNA damage with male cells showing relative resistance, whereas female cells exhibited increased sensitivity and impaired stress adaptation.
Conclusion: Sirt3 acts as a tumor suppressor in ER-α^+ and TNBC models by modulating mitochondrial metabolism and cellular stress responses. Its depletion causes DNA damage responses in a sex-dependent manner, emphasizing its role not only in cancer biology but also in aging and cellular senescence. These findings highlight the therapeutic potential of targeting Sirt3 in both cancer and age-associated disorders.

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L37

Investigation of individual radiosensitivity of prostate cancer patients under radiation treatment

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Keywords: Apoptosis, DNA damage, MicroRNome, Transcriptome, Radiotherapy, Radiation toxicity

Radiotherapy plays a central role in the treatment of patients with prostate cancer. One of the main challenges in modern radiation oncology is to develop efficient predictive models for the estimation of the radiation toxicity risk to tailor radiation treatment to the ndividual patient, improve the therapeutic success, and minimize severe adverse effects in cancer survivors. Numerous treatment-related, patient-specific, clinical, and biological factors, including genetic and epigenetic factors, as well as mediators of inflammation and immune response, may affect the possible risk of developing acute and late genitourinary and gastrointestinal radiation toxicity in prostate cancer patients undergoing radiotherapy. The multifactorial biological background of each cancer patient is responsible for individual differences in radiation-induced normal tissue toxicity. The investigation of the sensitivity of patient-derived peripheral blood mononuclear cells (PBMC) to ionizing radiation using different cell-based functional assays in combination with molecular mechanisms underlying this signature and specific acute and late radiation toxicity adverse events in prostate cancer patients using genomic, transcriptomic, epigenomic, and proteomic profiling is essential for identification of novel biomarkers and models for individual radiosensitivity assessment. The radiobiology research conducted within the Horizon Europe Twinning project RadExIORSBoost at the Institute for Oncology and Radiology of Serbia (coordinator) in collaboration with top-class leading European research institutions: Medical Faculty Mannheim (Heidelberg University), Medical University of Vienna, University of Leicester, and Institute of Oncology Ljubljana, aims to examine the multiple biological factors underlying individual normal tissue radiosensitivity in prostate cancer patients treated with radiotherapy. The systems biology approach is employed to explore the functional and molecular predictors of radiation toxicity using radiation-induced apoptosis rates of T lymphocytes, single cell gel electrophoresis and gamma-H2AX assays to measure radiation-induced DNA strand breaks and repair kinetics in PBMCs, in addition to transcriptome and miRNome profiling of PBMCs. Development and clinical validation of machine learning models for predicting individual radiosensitivity by combining individual, clinical, and biological factors are essential for a personalized radiotherapy approach.

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SESSION 1

BENEFITS OF COMBINATION THERAPIES IN CANCER TREATMENT

P01

Identification of Dual-Acting HDAC Inhibitors for Pancreatic Cancer Treatment through Drug Synergy Predictions and Molecular Modeling

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Keywords: drug design, polypharmacology, antineoplastic agents

Background: Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive and lethal cancers, with chemoresistance playing a significant role in its poor prognosis. This highlights the urgent need for the development of novel therapeutic strategies to overcome resistance and improve treatment outcomes for PDAC. Considering the widespread epigenetic alterations in PDAC, targeting epigenetic regulators such as histone deacetylases (HDACs) with inhibitors is a promising approach, especially in combination therapies. **Material and Methods:** In this study, we developed a bioinformatic screening protocol to predict potential synergistic combinations of HDAC inhibitors, including sirtuin (SIRT) inhibitors, by utilizing data on drug sensitivity and basal gene expression of pancreatic cancer cell lines. **Results and Conclusions:** Experimental validation in two pancreatic cancer cell lines, MIA PaCa-2 and PANC-1, confirmed synergy between HDAC inhibitors and either the sphingosine-1-phosphate (S1P) receptor agonist fingolimod or the Rho-associated protein kinase (ROCK) inhibitor RKI-1447. The bioinformatic screening also identified several previously unknown interaction partners for HDAC inhibitors, including ROCK, aurora kinase A (AURKA), glutaminase 1(GLS1) and WEE1 kinase inhibitors. These identified interactions were further investigated using structure-based molecular modeling to develop novel dual-acting HDAC inhibitors (HDAC/ROCK, SIRT/AURKA, HDAC/GLS1 and HDAC/WEE1). The molecular docking simulations revealed strong binding affinities of the novel dual-acting HDAC inhibitors to the respective targets. The key structural features responsible for the inhibition of HDAC1/4/6/8, SIRT, ROCK1, ROCK2, AURKA, GLS1 and WEE1 were identified and served as the basis for the development of these dual-acting HDAC inhibitors. Together with the predictions of drug synergies, these inhibitors prove to be promising candidates for future experimental validation. The most promising dual-acting HDAC inhibitors identified in silico will be synthesized for further in vitro enzyme and cell-based assays.

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P02

Inhibition of the membrane transporters in 5-fluorouracil-resistant colorectal cancer cells by chlorogenic acid

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Keywords: ABC transporters, resistance, 5-fluorouracil

Background: In the case of colorectal cancer, one of the most common and deadliest cancers, the commonly used chemotherapeutic agent is 5-fluorouracil (5-FU). However, a problem facing modern medicine is the development of resistance to chemotherapy in tumor cells. In several studies, chlorogenic acid (CHA), found in green tea and coffee extracts, has demonstrated a wide range of pharmacological actions, including anticancer effects. In our study, the potential of CHA to reverse the sensitivity of 5-FU-resistant colorectal cancer cells and inhibit the expression of key membrane transporters involved in the resistance development was investigated.
Material and Methods: Human colon carcinoma (HCT-116) cell lines, resistant to 5-FU, were used as a model system. The cytotoxicity of 5-FU and the achieved resistance were determined using an MTT assay and expressed as IC50 values and resistance factors for 24 h and 72 h. Effects of CHA on the expression of different ABC (ATP Binding Cassette) membrane transporters expressed in colon tissue were determined in HCT-116 5-FU resistant cells by qPCR methods.
Results: Results show that CHA has the potential to inhibit the expression of some transporters on the transcriptional level in 5-FU resistant HCT-116 cells. The significant result is the reduced relative expression of MRP5 and MRP8 membrane transporters in resistant cells treated with CHA, considering that these transporters were included in resistance development (their expression was increased in resistant cells compared to parental cells in our results).
Conclusions: According to its inhibitory activity on expression of the key membrane transporters in the emergence of 5-FU resistance in HCT-116 cells, chlorogenic acid should be further investigated for potential use to re-enhance the sensitivity of resistant cells to chemotherapeutics and inhibit their efflux. The effects on ABC transporter activity and more detailed experiments still need to be evaluated.

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P03

The impact of silibinin on the expression of genes involved in the process of apoptosis in malignant cells treated with selective COX-2 inhibitor

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Keywords: lung neoplasms; cyclooxygenase 2; celecoxib; silybin; apoptosis; carcinogenesis

Background: Chronic inflammation and increased activity of the enzyme cyclooxygenase-2 (COX-2) are recognized as important factors in the etiopathogenesis of lung cancer. Celecoxib, a selective COX-2 inhibitor, is being considered as a potential agent in the prevention and therapy of lung cancer. The aim of this study was the in vitro determination of the cytotoxic effect of celecoxib and silibinin (silybin), a natural flavonolignan isolated from the plant Silybum marianum, on the human cell line of lung adenocarcinoma (A549), as well as an analysis of the expression of genes involved in inflammatory and apoptotic cell responses.
Material and Methods: The cytotoxicity of celecoxib and silibinin on A549 cells was evaluated using dye exclusion and MTT assays. Quantification of gene expression was performed using the quantitative real-time polymerase chain reaction with reverse transcription (qRT-PCR), and was analyzed using the comparative ΔΔCt method, with ACTB as a

reference gene. Statistical processing of the results was done by one-factor analysis of variance (ANOVA) with Taki's post-hoc test.
Results: Celecoxib and silibinin exhibit a concentration-dependent increase in cytotoxicity i.e. inhibition of viability and proliferation of A549 cells. Compared to the untreated, control group, celecoxib decreased the expression of the gene responsible for the synthesis of the COX-2 isoenzyme (PTGS2) and the expression of the antiapoptotic BCL2 gene ($p<0.01$), and increased the expression of the tumor suppressor gene TP53 and the proapoptotic BAX gene, without statistical significance. The addition of silibinin to celecoxib showed a synergistic effect in further increasing the mRNA levels of TP53 and BAX genes ($p<0.01$).
Conclusions: COX-2 is an important factor in the survival and proliferation of malignant cells. Celecoxib and silibinin show antiproliferative and proapoptotic effects on lung cancer cells. The addition of silibinin to celecoxib offers the potential to use lower doses of celecoxib to exert its antineoplastic effects, thereby reducing the side effects of this selective COX-2 inhibitor.

P04

Investigation of the effect of exportin-1 gene inhibition with selinexor on glioma

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Keywords: drug resistance, exportin 1, glioma, temozolomide

Background: Glioblastoma (GBM) is the most common malignant brain tumor, typically treated with surgery followed by temozolomide (TMZ) chemotherapy and radiotherapy. However, recurrence and therapy resistance are common, highlighting the need for new treatment strategies. Exportin 1 (XPO1), a nuclear export protein, translocates tumor suppressors, regulatory proteins, and RNA from the nucleus to the cytoplasm. Dysregulated nuclear export contributes to tumor progression and chemoresistance. Understanding the role of XPO1 in GBM and exploring its inhibition could offer new therapeutic opportunities.
Materials and Methods: TMZ-resistant GBM cell line LN18, standard GBM cell line U87MG, and TMZ-sensitive LN229 cell line were used as in vitro models. The selective XPO1 inhibitor Selinexor was administered alone and in combination with TMZ. The effects on cell viability were assessed, along with mechanistic evaluations including apoptosis, cell cycle distribution, colony formation capacity, and expression levels of genes involved in DNA repair and resistance.
Results: The combination of Selinexor and TMZ was effective in reducing cell viability across all cell lines, with the most dramatic effect observed in the TMZ-resistant LN18 cells. Mechanistic analysis showed that cell death in resistant cells occurred primarily through apoptosis, while TMZ-sensitive and non-resistant lines (U87MG and LN229) exhibited G1/G0 and S cell cycle arrest. Colony formation assays revealed that combination treatment significantly reduced the number of colonies, indicating impaired long-term proliferative capacity. Furthermore, in TMZ resistant cells, the combination treatment downregulated genes involved in DNA damage response (DDR), mismatch repair (MMR), and other resistance-related pathways. This suggests that XPO1 inhibition may enhance TMZ efficacy in resistant cells by suppressing key resistance mechanisms.
Conclusions: Targeting XPO1 with Selinexor may potentiate the effect of TMZ, particularly in drug-resistant GBM models. These findings support the combination's potential to overcome resistance and reduce tumor recurrence, warranting further investigation in preclinical and clinical studies.

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SESSION 2

PERSONALIZED ANTICANCER TREATMENT THROUGH TARGETING SIGNALING PATHWAYS

P05

TFEB Controls Chemoresistance in NSCLC

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Keywords: ABCA1, MDR, NSCLC, TFEB

Background: In NSCLC, the effectiveness of chemo-immunotherapy is influenced by the dysregulated expression of ABC transporters, including the overexpression of ABCC1 and the reduced expression of ABCA1. While ChIP-Seq studies in endothelial cells have identified several ABC transporters as transcriptional targets of transcription factor EB (TFEB), its role in cancer remains unclear. TFEB, a leucine zipper protein, is a key regulator of lysosomal biogenesis and autophagy, and emerging evidence suggests its involvement in modulating the immune recognition of cancer cells by the host immune system. In this study, we investigated if TFEB affects the response to chemotherapy and to Vγ9δ2 T- lymphocytes in non-small cell lung cancer (NSCLC).
Materials and Methods: The influence of TFEB/ABCC1/ABCA1 expression on the survival of NSCLC patients was examined using data from the TCGA-LUAD cohort and a retrospective cohort from our institution. Human NSCLC cells with TFEB silencing (shTFEB) were assessed for ABC transporter expression, chemosensitivity, and immune-mediated cytotoxicity. The chemo-immuno-sensitizing effects of nanoparticles encapsulating zoledronic acid (NZ) on shTFEB tumors and the tumor immune microenvironment were investigated in Hu-CD34+ mice through single-cell RNA sequencing.
Results: TFEBlowABCA1lowABCC1high phenotype proved to be the worst prognosis for the NSCLC patients. ChIP assay indicated that ABCA1 is a direct target of TFEB. By reducing the pERK1/2, shTFEB cells had reduced ERK-1/2-mediated activation of SREBP2, which modulates genes of cholesterol homeostasis. As such, TFEB silencing down-regulated genes of cholesterol synthesis, decreased expression and activity of the cholesterol/IPP transporter ABCA1, the efflux of IPP, and the NSCLC killing by Vγ9δ2 T-lymphocytes. In parallel, shTFEB cells had increased expression of ABCB1 and ABCC1 and significantly higher IC50 values for cisplatin. The results of immune xenografts confirmed that shTFEB tumors were more resistant to cisplatin than wild-type counterparts. The combination of cisplatin and NZ was effective in reversing the chemo-immuno-resistance of shTFEB tumors.
Conclusions: This study revealed that TFEB plays a crucial role in regulating sensitivity to chemotherapy and immune-mediated killing in NSCLC. Incorporating TFEBlowABCA1lowABCC1high signature into the diagnostic workflow could provide valuable insights for optimizing treatment selection on an individual basis in NSCLC patients.

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P06

Foxo3a mediates Sirt3-dependent regulation of ERα signaling in MCF-7 breast cancer cells

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Keywords: Sirtuin 3, Foxo3a, MCF-7, breast cancer, estrogen receptor

Background: Breast cancer remains the most frequently diagnosed cancer among women, with approximately 70% of cases being estrogen receptor alpha (ERα) positive. Estrogen (E2) plays a critical role in the development and progression of these hormone-dependent tumors. Sirtuin 3 (Sirt3), a major mitochondrial deacetylase, has shown context-dependent roles in cancer, acting as either an oncogene or a tumor suppressor. In estrogen/progesterone receptor-positive MCF-7 breast cancer cells, we previously demonstrated that Sirt3 overexpression increases ERα levels while reducing tumorigenic properties through upregulation of p53 and attenuation of E2 signaling. In this study, we explore the potential involvement of transcription factor Foxo3a as a mediator in the Sirt3–p53–ERα regulatory axis. Foxo3a acts as a tumor suppressor in multiple cancer types, although its specific role in cancer remains unclear. Moreover, it shows promise as a potential marker for tumor diagnosis and prognosis in breast cancer patients.
Materials and methods: We use MCF-7 breast cancer cells transfected with the FLAG-tagged Sirt3 (MCF-7S3) or empty pcDNA3.1 plasmid (MCF-7C). Following a combination of Sirt3 overexpression, E2 and fulvestrant (ICI) treatments, we analyzed the morphology and survival of the cells, protein expression and interaction by Western blot and co-immunoprecipitation and examined localization using confocal microscopy.
Results: Besides observed tumor-suppressive effects of Sirt3 in these cells, we revealed that Foxo3a expression and localization are modulated by both Sirt3 and hormonal stimuli. Co-immunoprecipitation experiments confirmed an interaction between Foxo3a and ERα, which was significantly reduced in Sirt3-overexpressing clones compared to controls, and almost completely reduced in both clones upon E2 treatment.
Conclusions: Sirt3 expression induces p53 and Foxo3a expression and impacts their localization in MCF-7 breast cancer cells, coordinately stimulating tumor suppressor cellular processes in tumor cells. Both Sirt3 and E2 disrupt Foxo3a–ERα interaction, potentially shifting the function of Foxo3a away from ERα-regulated transcription toward p53-mediated tumor suppression. All these results indicate that Foxo3a acts as a functional mediator of Sirt3-driven changes in ERα signaling in MCF-7 cells, highlighting the Sirt3–Foxo3a–p53 axis as a potential target in the treatment of hormone-dependent breast cancer.

P07

Exploring HPV E6 interaction network: new insights into HPV-induced tumorigenesis

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Keywords: HPV, cancer, interactome

Background: While most people will be infected with Human papillomaviruses (HPVs) at some stage of their lives, only persistent infection with high-risk (HR) types, such as HPV 16 and 18, can lead to the development of various cancers. Although HR types are predominantly linked to cervical cancer, they are also associated with a subset of anogenital and head and neck cancers. In contrast, low-risk (LR) HPV types, such as HPV 6 and 11, are not considered tumorigenic, as they primarily cause benign warts. The hallmark of HPV-induced tumorigenesis is uncontrolled expression of two main viral oncoproteins, E6 and E7, which target multiple cellular regulators, including p53 and pRB, the major tumor suppressor proteins involved in the control of the cell cycle and apoptosis. Furthermore, HR E6 oncoproteins also interact with a number of PDZ-domain containing proteins, which act as cellular tumor suppressors and are involved in cell polarity regulation. E6s bind to these targets via their PDZ-binding motifs (PBMs), present on their extreme C-termini, ultimately driving their degradation in the proteasome/dependent manner. In this study we identified a novel E6 interacting partner, which has been recently shown to be strongly associated with promotion and progression

of a number of human cancers.
Materials and Methods: The major experimental approaches involved cell culture models, which were the basis for protein interaction assays, such as GST pull-down assays, degradation assays in the presence of proteasomal inhibitors, mutational analysis, and investigation of the specificity of HPV E6 type in targeting substrate.
Results: Our preliminary data demonstrate that HR E6 oncoproteins (16, 18, 33) interact with the novel cellular substrate in a PBM/PDZ dependent manner with different intensities. Here we show that this interaction results in a significant reduction in the protein expression levels of the novel interacting partner. Notably, the downregulation was not observed in the presence of the proteasome inhibitor, implying that this PBM/PDZ-dependent interaction is likely regulated by the proteasome.
Conclusions: Thus, our results provide new insights into the complexity of the E6 interactome and are likely to reveal important novel mechanisms by which HPV contributes to malignant transformation.

P08

Silencing of NaV1.7 voltage-gated sodium channel by siRNA modulates oncogenic pathways in pancreatic cancer

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Keywords: apoptosis, cell proliferation, neoplasm invasiveness, pancreatic neoplasms, RNA interference, voltage-gated sodium channels

Background: Pancreatic cancer (PCa) is one of the most aggressive malignancies globally, currently ranking fourth in cancer-related deaths in the United States and seventh worldwide. Despite advances in treatment, the 5-year survival rate remains below 10%, largely due to late diagnosis, limited biomarkers, an immunosuppressive tumor microenvironment, intrinsic chemoresistance, and high metastatic potential (1–4). Gemcitabine, the current first-line chemotherapeutic, offers only modest survival benefits due to rapid resistance development (5,6). Thus, novel therapeutic targets and combination strategies are urgently needed. Voltage-gated sodium channels (VGSCs), initially known for roles in excitable tissues, are now implicated in cancer progression, including proliferation, invasion, and metastasis in several epithelial cancers (7,8). The NaV1.7 isoform (SCN9A) is of particular interest due to its elevated expression in metastatic tumors and its influence on oncogenic signaling (9). Although tumor database analyses suggest NaV1.7 is overexpressed in pancreatic ductal adenocarcinoma (PDAC), its functional role in PCa remains unexplored. Based on our prior VGSC work in breast and pancreatic cancer (10), we investigated whether siRNA-mediated NaV1.7 silencing could inhibit key malignant features of PCa and enhance gemcitabine efficacy, aiming to establish NaV1.7 as a potential novel therapeutic target.
Materials and Methods: Human keratinocyte cell line HaCaT (control) and pancreatic cancer cell lines PANC-1 and MiaPaCa-2 (PDAC models) were cultured in DMEM with 10% FBS at 37°C and 5% CO_2. Targeted gene silencing of NaV1.7 (SCN9A) adult and neonatal isoforms was performed using specific siRNAs transfected via Lipofectamine 2000 (Invitrogen). Knockdown efficiency was confirmed by qRT-PCR and Western blot. Cell proliferation was assessed with MTS assays (Promega) at 24, 48, and 72 hours post-transfection. Colony formation was evaluated after 10–14 days in 6-well plates treated with siRNAs and/or gemcitabine (Gemzar®). In PANC-1 cells, combinatorial treatments with sub-cytotoxic gemcitabine and NaV1.7 siRNAs were tested for synergistic effects. Invasion and migration were analyzed via Transwell assays (Matrigel-coated and uncoated), and cell motility was assessed using wound healing assays. Apoptosis and cell cycle distribution were evaluated by flow cytometry using Annexin V-FITC/PI staining. mRNA expression was quantified by qRT-PCR (SYBR Green), and protein levels of key signaling molecules (P-Src, P-FAK, Integrin β1, EF2K, P-Akt, P-mTOR, cleaved PARP, Caspase-3/9, Cyclin D1/E1) were analyzed by Western blot. All experiments were conducted in triplicate. Statistical analysis was performed using one-way ANOVA (Tukey's post-hoc), with $p < 0.05$ considered significant.
Results: Quantitative real-time PCR (qRT-PCR) analyses confirmed that both adult and neonatal isoforms of NaV1.7 (SCN9A) mRNA were significantly overexpressed in the pancreatic cancer cell lines PANC-1 and MiaPaCa-2 compared to the non-cancerous HaCaT cell line ($p < 0.01$). Western blot analysis further validated

that NaV1.7 protein levels were markedly elevated in both pancreatic cancer cell lines. Following siRNA-mediated knockdown, a substantial reduction in both NaV1.7 mRNA and protein expression was achieved in PANC-1 and MiaPaCa-2 cells (>80% downregulation, $p < 0.001$), confirming the high efficiency of gene silencing. Functionally, NaV1.7 knockdown resulted in a significant decrease in pancreatic cancer cell proliferation, as assessed by MTS assays conducted at 24, 48, and 72 hours post-transfection ($p < 0.001$ at all time points compared to controls). Importantly, NaV1.7 siRNA treatment did not elicit any cytotoxic effect in normal HaCaT cells, indicating a tumor-specific impact. In clonogenic assays, the ability of PANC-1 and MiaPaCa-2 cells to form colonies was dramatically suppressed following NaV1.7 silencing, with an approximately 70–75% reduction in colony numbers relative to negative controls ($p < 0.001$). In PANC-1 cells, combination treatments with sub-cytotoxic doses of gemcitabine and NaV1.7 siRNA demonstrated a clear synergistic effect, significantly enhancing the cytotoxicity of gemcitabine (combination index CI < 1). The combined treatment led to further suppression of proliferation and clonogenic potential beyond either treatment alone ($p < 0.001$). Cell invasion and migration assays revealed that NaV1.7 siRNA treatment significantly impaired the invasive capacity of both PANC-1 and MiaPaCa-2 cells, with a >60% reduction in invasion through Matrigel-coated Transwell inserts ($p < 0.001$). Similarly, migration was decreased by >50% following NaV1.7 knockdown ($p < 0.001$). In wound-healing assays, NaV1.7 silencing delayed wound closure kinetics by more than 60% at 24 hours compared to untreated controls, indicating marked inhibition of cancer cell motility. Flow cytometric analysis demonstrated that NaV1.7 silencing induced robust apoptosis in both pancreatic cancer cell lines, with significant increases in early and late apoptotic populations (Annexin V+/PI+ cells, $p < 0.001$). Moreover, NaV1.7 knockdown caused a pronounced arrest in the G1 phase of the cell cycle, with a corresponding decrease in S-phase populations ($p < 0.001$), suggesting impaired cell cycle progression. At the molecular level, Western blot analyses showed significant downregulation of key proteins associated with proliferation (Cyclin D1, Cyclin E1), survival (P-Akt, P-mTOR), and invasion/migration (P-Src, P-FAK, Integrin β1, EF2K) following NaV1.7 knockdown in both cancer cell lines. In addition, markers of apoptosis, including cleaved PARP and cleaved Caspase-3/9, were markedly elevated, further supporting the pro-apoptotic effect of NaV1.7 inhibition. Collectively, these results demonstrate that NaV1.7 channel activity critically regulates multiple hallmarks of pancreatic cancer, including proliferation, invasion, migration, apoptosis, and drug resistance, and that its silencing offers a promising therapeutic approach.

Conclusions: Our study provides the first comprehensive and mechanistic evidence that both adult and neonatal isoforms of the NaV1.7 voltage-gated sodium channel play a critical role in driving pancreatic cancer progression, metastatic potential, and therapeutic resistance. siRNA-mediated targeting of NaV1.7 not only effectively suppressed key malignant phenotypes—including proliferation, clonogenicity, invasion, migration, and survival—but also significantly enhanced the cytotoxic efficacy of the current first-line chemotherapeutic agent, gemcitabine, in pancreatic cancer cells. Importantly, these effects were observed without inducing cytotoxicity in normal keratinocyte cells, highlighting the tumor-specific therapeutic potential of NaV1.7 inhibition. Furthermore, our findings revealed that NaV1.7 silencing modulates multiple oncogenic signaling pathways involved in cell cycle regulation, survival, and metastasis, thereby providing mechanistic insights into its role as a key regulator of pancreatic cancer biology. These collective results position NaV1.7 as a highly promising and novel molecular target for therapeutic intervention in pancreatic cancer. Given the urgent unmet clinical need for more effective treatment strategies in this aggressive malignancy, further validation of these findings in preclinical in vivo models and translational studies is warranted. Ultimately, the development of NaV1.7-targeted therapies, either as monotherapy or in combination with standard chemotherapeutics, may offer new avenues to improve patient outcomes in pancreatic cancer.

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P09

From *in silico* to clinical validation: CYBB and IDO1 as potential prognostic markers in rectal cancer

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Keywords: locally advanced rectal cancer; neoadjuvant chemoradiotherapy; prognostic biomarkers; CYBB; IDO1; disease-free interval

Background: Locally advanced rectal carcinoma (LARC) is typically treated with neoadjuvant chemoradiotherapy (nCRT) followed by surgery. Identifying predictive and prognostic biomarkers is crucial for selecting patients who will benefit the most from neoadjuvant treatment. In our previous study, an in silico approach was used to select candidate genes associated with response to nCRT [1]. Three genes involved in the Hallmark inflammatory response pathway and associated with immune evasion (IL6, CXCL9, and CYBB) were selected for further validation, along with IDO1, a gene identified through literature review as having potential relevance [2,3]. The expression of these genes was measured using quantitative real-time PCR (qRT-PCR) in pretreatment formalin-fixed paraffin-embedded (FFPE) tissue samples, using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a reference. However, in a prospective cohort from the Institute for Oncology and Radiology of Serbia, no significant correlation was observed between the expression of the selected genes (IL6, CYBB, CXCL9, IDO1) and overall treatment response [1]. Patients were divided into responders—those who achieved a clinical complete response (cCR) and were managed with a "watch and wait" approach, as well as those with tumor regression grade (TRG) 1 or 2 according to Mandard, in patients who underwent surgery. The non-responder group included patients with TRG 3 or 4. In the subgroup of patients who underwent surgery, a comparison between the best responders (TRG1) and those with the poorest response (TRG4) revealed a statistically significant difference ($p<0.05$) in the expression of the IDO1 gene [1]. Higher expression of IDO1 was associated with a better treatment response [1]. The CYBB gene encodes the gp91-phox subunit of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex, which is primarily recognized for its role in the oxidative burst of the immune system. A literature review of the CYBB gene revealed a statistically significant association between its expression levels and survival in patients with LARC, with higher expression linked to improved overall survival ($p = 0.02$) [4]. However, emerging studies suggest that CYBB activity is also associated with oxidative stress and the response to radiotherapy in cancer treatment [5]. Elevated oxidative stress levels have been linked to increased radiosensitivity of cancer cells. The prognostic significance of IDO1 has been demonstrated in several malignancies. In renal cell carcinoma, high IDO1 expression correlates with poorer outcomes, whereas in ovarian cancer and melanoma, it has been identified as a favorable prognostic marker [4]. Similarly, CXCL9 has shown prognostic relevance in renal, endometrial, breast, and ovarian cancers, where its elevated expression is associated with better survival [4]. In colorectal cancer (CRC) specifically, increased CXCL9 expression has been linked to a favorable prognosis in a cohort of 326 patients [6]. Regarding IL6, literature data highlight its involvement in the development and progression of CRC [7], as well as its potential as a prognostic biomarker. While some studies have reported that elevated plasma IL6 levels are associated with poorer survival in CRC patients, these findings have not been consistently validated in subsequent research [8]. Although investigating genes associated with the inflammatory response holds considerable promise for understanding therapy response in rectal cancer, the lack of validation in our study may be attributed to population-specific factors and the limited sample size [1]. Since their predictive significance was not confirmed in previous research, this study aimed to evaluate their prognostic role in our cohort of patients with LARC.

Patients and Methods: Between June 2020 and May 2022, we prospectively enrolled 82 patients with LARC who

underwent long-course nCRT. Radiation therapy was delivered using volumetric modulated arc therapy with a simultaneous integrated boost (VMAT-SIB), combined with concomitant chemotherapy (5-fluorouracil and leucovorin) administered during the first and fifth weeks of treatment. Treatment response was evaluated eight weeks after the completion of nCRT. Patients who achieved a cCR and had tumors located distally (not amenable to sphincter-sparing surgery) were not immediately referred for radical surgery but were instead enrolled in a strict follow-up program (i.e., the "watch and wait" approach). In cases of tumor regrowth, salvage surgery was performed. Following surgery, the follow-up protocol included a first surveillance colonoscopy at 12 months post-treatment, then every 3–5 years depending on findings. Chest, abdominal, and pelvic CT scans were performed every 6 months for the first 3 years, and annually thereafter. Clinical evaluations and tumor markers (CEA, CA 19-9)were measured every 3–6 months in the first 3 years, and then every 6–12 months Overall survival (OS) was defined as the time from diagnosis to the date of last clinical follow-up or death. Disease-free interval (DFI) was calculated from the date of surgery or, for patients in the "watch and wait" group without relapse, from the control MRI until disease progression, death, or last follow-up. For patients included in the "watch and wait" protocol who experienced tumor regrowth during follow-up, DFI was calculated from the date of salvage surgery until disease progression, death, or last follow-up. Gene expression levels (IL6, CXCL9, CYBB, IDO1) were measured using qRT-PCR in pretreatment FFPE samples. Patients were divided into two groups based on DFI status (with or without disease relapse), and gene expression levels were compared between the groups.

Results: Of the 82 enrolled patients, 65 (79.3%) underwent surgery, while 17 (20.7%) were managed with the "watch and wait" strategy. Among the "watch and wait" cohort, 8 patients experienced tumor regrowth; 7 of these (87.5%) underwent successful salvage surgery. One patient developed both local and distant relapse and was not operated on. The median disease-free interval (DFI) was 36 months (range: 1–54 months), with a 3-year DFI rate of 74.8%. Median overall survival (OS) was 46 months (range: 12–58 months), with a 3-year OS rate of 88.9%. When comparing gene expression between patients with and without disease relapse, statistically significant differences were observed for CYBB and IDO1 ($p < 0.05$). Receiver operating characteristic (ROC) curve analysis was performed to determine the optimal cut-off values for these genes. While the ROC analysis itself did not show statistical significance (CYBB: $p = 0.744$, AUC = 0.670; IDO1: $p = 0.544$, AUC = 0.679), when the derived cut-off values were applied, higher CYBB expression (cut-off value 0.447) was significantly associated with better DFI, while higher IDO1 expression (cut-off value 1.471) was significantly associated with better both DFI and OS ($p < 0.05$ for both).

Conclusions: This prospective study evaluated the prognostic significance of selected immune-related genes in patients with locally advanced rectal cancer treated with neoadjuvant chemoradiotherapy. While initial in silico findings did not correlate with treatment response, further analysis revealed that high expression levels of CYBB and IDO1 were significantly associated with disease-free and overall survival. These results suggest that CYBB and IDO1 may serve as valuable prognostic biomarkers, supporting their potential role in risk stratification and individualized patient management using a multigene prognostic panel. Further validation in larger, independent cohorts is warranted.

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P10

Estrogen receptor modulation and Hedgehog-GLI crosstalk in Head and neck squamous cell carcinoma

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Keywords: squamous cell carcinoma of head and neck; receptors, estrogen; arsenic trioxide; tamoxifen

Background: Head and neck squamous cell carcinoma (HNSCC) is a heterogeneous malignancy of the upper aerodigestive tract. Despite therapeutic progress, it remains associated with high mortality and impaired quality of life. Key risk factors include tobacco, alcohol, and HPV infection. A higher male prevalence suggests a role for sex hormones, notably estrogen and its receptors, in modulating disease risk, possibly due to male-specific risks or female hormonal protection. To elucidate the involvement of estrogen receptors (ER) in HNSCC pathogenesis, we performed a comprehensive analysis of both nuclear and membrane-associated estrogen receptors, with particular emphasis on their interaction with the Hedgehog-GLI signaling pathway.
Materials and Methods: Using quantitative real-time PCR (qPCR), we analyzed the mRNA expression levels of ESR1, ESR2, GPER1, and SCN2A in 93 primary HNSCC tumors, 26 positive lymph nodes, and 42 healthy oral mucosa samples. We then compared ER expression patterns with clinical parameters, including patient age, sex, tumor stage and grade, HPV status, and primary tumor site. Given the differential expression of ESR1 and ESR2 in tumor versus normal tissues, we investigated the impact of modulating ER activity using estradiol and tamoxifen, along with inhibition of the Hedgehog-GLI pathway via arsenic trioxide, on cellular behavior in HNSCC.
Results: We established CAL-27 cell lines stably expressing ESR1 or ESR2 using CRISPR-Cas9-mediated gene editing. Tamoxifen treatment significantly reduced viability and colony formation in ESR1-KI cells, while ESR2-KI cells exhibited increased resistance compared to the parental line. Conversely, ESR2-KI cells showed enhanced sensitivity to arsenic trioxide and its combination with tamoxifen. Functional assays revealed that tamoxifen also reduced colony formation in ESR1-KI cells and migration capacity in both KI cell lines. Furthermore, single and combined treatments significantly reduced the number of cells in the G2/M phase of the cell cycle in both KI cell lines, while in the ESR2-KI cell line combined treatment increased the number of apoptotic cells.
Conclusions: In summary, our results suggest that elevated ESR1 and ESR2 expression influences the biological characteristics of HNSCC cells. Further investigations are required to fully elucidate their potential as biomarkers or therapeutic targets in HNSCC.

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P11

Sensitizing hepatocellular carcinoma (HCC) cells to lenvatinib via activation of the ferroptosis pathway: insight on SECTM1 as a target

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Keywords: drug resistance, hepatocellular carcinoma, lenvatinib

Background: The prognosis of hepatocellular carcinoma (HCC) remains poor due to late diagnosis, tumor recurrence, and drug resistance. Lenvatinib, an oral multi-kinase inhibitor, was approved for patients with unresectable HCC in 2018. However, the genetic heterogeneity of HCC tumors is highly associated with primary resistance. Previously, we identified novel genes associated with lenvatinib resistance by analyzing four public datasets and validated them in HCC cells. In this study, we aimed to determine the molecular mechanism of these genes that enables the cells to be sensitive to lenvatinib.
Materials and Methods: MTT assay was performed in HCC cells treated with ferroptosis activators (erastin and RSL3) for 24, 48, and 72 hours. Based on the IC50 values, two doses of the activators were selected for further experiments. Huh7 cells were treated with erastin (0.1µm and 1.0µm) and RSL3 (0.01µm and 0.05µm) for 24h. Five lenvatinib resistance genes were probed by RT-qPCR in HCC cells and in human tissue samples.
Results: MTT assay showed that Huh7 cells were more sensitive to ferroptosis activators than JHH6 cells. The different drug responses could be due to the inherent differences between the two cell lines, with Huh7 being less aggressive with lower metastatic potential and JHH6 cells being highly tumorigenic with greater metastatic potential. In Huh7 cells treated with lenvatinib, there was significant upregulation of SECTM1, IFI6, and NPC1L1. In Hep3B cells, all five genes (SECTM1, IFI6, NPC1L1, FBLN7 and SEZ6L2) were upregulated. Interestingly, in Huh7 cells treated with ferroptosis activators, there was downregulation of SECTM1, FBLN7, and NPC1L1. This suggests that the activation of ferroptosis sensitizes cells to lenvatinib, and this event is gene-dependent. Of the five candidate genes, SECTM1 expression was analyzed in 61 paired human HCC tissue samples. SECTM1 was significantly upregulated in tumor tissues compared with the adjacent normal liver tissue (p=0.001). High SECTM1 expression was associated with poor prognosis with overall survival probability of 45% vs 60% in low-expressing patients. There was higher SECTM1 expression in Grade 4 tumors compared to lower grades and in nodal metastasis.
Conclusions: The high expression of certain genes is associated with resistance to lenvatinib. Here, we show that sensitivity to lenvatinib can be achieved via activation of ferroptosis. Overall, our current study revealed SECTM1 as a possible therapeutic target.

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P12

The effect of PDK1 inhibition on human breast cancer cell growth

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Keywords: 3-phosphoinositide-dependent protein kinase 1; cell death; RNA, small interfering; triple negative breast neoplasms

Background: Phosphoinositide-dependent kinase-1 (PDK1) is a major serine/threonine kinase that regulates various cellular processes including survival, proliferation, and migration through the PI3K/AKT/mTOR and MAPK/RSK pathways.

In this study, we aimed to investigate the effect of both pharmacological and genetic inhibition of PDK1 on human breast cancer cell proliferation and viability.
Methods: Three breast cancer cell lines (MCF7, SK-BR-3 and MDA-MB-231) were screened using four small-molecule PDK1 inhibitors (GSK2334470, BX-517, BX-912, and PDK1- IN-RS2) via SRB cytotoxicity assay. The triple-negative MDA-MB-231 cell line was selected for further experiments. The most active inhibitor was then tested in wound healing (scratch) assay and cell viability assay in order to investigate its anti-migratory and anti-proliferative capacity. Flow cytometry was performed to examine the inhibitor's effect on the cell cycle of asynchronous MDA-MB-231 cells, while siRNA-mediated silencing of PDK1 was used to compare pharmacological versus genetic inhibition of the kinase. Western blotting was used to evaluate the expression of apoptotic markers, including caspases, in PDK1-silenced MDA-MB-231 cells.
Results: Among all tested inhibitors, BX-912 exhibited dose- and time-dependent suppression of proliferation and migration of MDA-MB-231 cells without inducing notable cytotoxicity, suggesting a cytostatic effect. In contrast, silencing of PDK1 using specific siRNA induced significant cell death at both 24 and 48 hours post-transfection, partially through apoptotic pathways, suggesting that additional mechanisms of cell death may also be involved.
Conclusions: Our results suggest that while pharmacological inhibition of PDK1 suppresses growth and migration in breast cancer cells, genetic silencing using siRNA leads to a stronger and more profound cytotoxic effect. This highlights both the importance of the role of PDK1 in the survival of Triple Negative Breast cancer cells and the need for more selective and potent PDK1 inhibitors. Further experiments are underway to investigate the role and therapeutic potential of PDK1 inhibition in breast cancer.

P13

Sigma receptor 2 ligand Siramesine: Unraveling its role in angiogenesis

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Keywords: angiogenesis, siramisine, σ2 receptor ligand, CAM assay, anti-angiogenic effect

Background: Siramesine is a selective σ2 receptor ligand that has demonstrated potent antiproliferative properties; however, its role in angiogenesis remains unexplored. This study investigated the antiangiogenic and anticancer potential of siramesine using in vitro, ex vivo, and in vivo models.
Methods: The cytotoxic effect of siramesine on endothelial cells (EA.hy926) was examined with the SRB assay. Molecular markers of angiogenesis and survival pathways were also examined using Western blotting. The anti-angiogenic activity of siramesine was evaluated by an in vitro tube formation assay, an ex-vivo aortic ring assay, and in vivo chick embryo chorioallantoic membrane (CAM) and Matrigel plug assay.
Results: Cytotoxicity assays (SRB) revealed dose- and time-dependent suppression of endothelial cell viability in HUVEC and EA.hy926 cell lines. Functional assays, including tube formation, wound healing, colony formation and aortic ring assays, demonstrated that siramesine significantly inhibited angiogenic activity and cellular migration. Western blot analysis showed that siramesine modulates angiogenesis-related pathways by downregulating PGRMC1 and altering AKT and ERK1/2 signaling in both endothelial cells and PDAC xenograft tissues. Additionally, siramesine induced G1 cell cycle arrest, as evidenced by FACS analysis. In vivo validation via CAM and Matrigel plug assays confirmed the presence of strong antiangiogenic effects.
Conclusions: Taken together, these findings suggest that siramesine exerts potent anti-angiogenic and anti-tumour effects by modulating survival and proliferation pathways, thus supporting its potential as a novel therapeutic candidate in PDAC. Further mechanistic studies are required to elucidate its precise mode of action.

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P14

Studying the role of Sigma 2 receptor TMEM97 and PGRMC1 in the progression of Pancreatic ductal adenocarcinoma

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Keywords: pancreatic neoplasm, progesterone receptor membrane component 1, Siramesine, small interfering RNA, transmembrane protein 97

Background: Pancreatic cancer remains one of the most lethal malignancies. TMEM97 and PGRMC1, components of the sigma-2 receptor complex, have been linked to cancer progression and therapy. This study explores their role in primary PDAC cells, focusing on proliferation, migration, and response to the sigma-2 ligand siramesine. **Materials and Methods:** Primary PDAC cells (EANM13_att) were used. TMEM97 and PGRMC1 were silenced via siRNA, with protein knockdown confirmed by Western blot. SRB assays assessed Siramesine's cytotoxic and antiproliferative effects. Clonogenic assays examined long-term proliferation. Migration was tested via transwell assays. Flow cytometry analyzed cell cycle distribution. In vivo tumorigenesis was studied using xenograft models with receptor-silenced cells. **Results:** Silencing TMEM97 and PGRMC1 using siRNA effectively reduced their protein expression. Clonogenic assays revealed that silencing both receptors significantly reduced the ability of cells to form colonies. Furthermore, PGRMC1-silenced cells exhibited significant spontaneous cell death, and migration assays indicated that PGRMC1 may be essential for the migratory capacity of pancreatic cancer cells. In vivo, the results showed that silencing TMEM97 reduces the tumorigenic capacity of PDAC cells, resulting in a delayed appearance and growth of tumors. Conversely, PGRMC1, silencing was not found to significantly affect tumor growth compared to control (scrambled siRNA) tumors. The cytotoxicity of siramesine was found to be independent of the status of both TMEM97 and PGRMC1 while flow cytometry demonstrated siramesine-induced G0/G1 arrest across all groups. **Conclusions:** Silencing PGRMC1 revealed that this receptor may significantly influence certain properties of pancreatic cancer cells in vitro, such as survival and, importantly, migration. Conversely, TMEM97 was found to have a significant impact on PDAC progression in vivo, delaying tumor appearance and growth. The results suggest that both PGRMC1 and TMEM97 are critical for various features of cancer cell positioning, making them potential therapeutic targets in pancreatic cancer. It is also noteworthy that the results of the current study suggest that Siramesine may act independently of the presence of the receptors.

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P15

Impact of high-risk HPV E6 oncoproteins on the Notch signalling pathway

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Keywords: HPV, oncoproteins, Notch signalling

Background: Human papillomaviruses (HPVs) comprise a diverse family of small DNA viruses that infect epithelial cells. Among the different HPV genera, alpha (α) and beta (β) types are of particular interest due to their relevance to human health. α-HPVs mainly infect mucosal tissues, with high-risk types such as HPV16 and HPV18 being key contributors to cervical cancer. β-HPVs are commonly linked to harmless skin infections, although specific β-types such as HPV5 and HPV8 have been identified as cofactors in the development of skin cancers. To enable viral survival and proliferation within host cells, both α- and β-HPVs employ their respective E6 oncoproteins, which subvert cellular processes through protein-protein interactions. For example, α-E6s utilize their LXXLL binding motif to hijack the E6AP ubiquitin-ligase in order to target the p53 tumour suppressor for proteasomal degradation. Using the same binding motif,

β-E6s preferentially bind MAML1, a transcriptional co-activator that plays a role in the Notch signalling pathway. This interaction inhibits the expression of the Notch signalling target genes, consequently slowing down the differentiation of infected keratinocytes. Notably, our recent analyses have shown that multiple α-E6s also bind MAML1, which results in increased E6 stability. In the current study, we aimed to broaden our understanding of the reported α-E6-MAML1 interaction and test whether it is also implicated in the inhibition of the Notch signalling pathway and keratinocyte differentiation.
Materials and Methods: GST pull-down and co-immunoprecipitation assays were employed to compare the binding affinities among the E6 proteins from HPV types 8, 16, and 18 towards MAML1. To verify the effects of 16 E6 and 18 E6 on the expression of Notch-responsive genes, luciferase assays and qPCR were performed, with 8 E6 serving as a positive control. Moreover, differentiation assays were carried out in keratinocytes that stably express the abovementioned E6 proteins.
Results: According to our data, cells exhibited reduced expression of Notch-responsive genes in the presence of MAML1-binding α-E6s. Likewise, the expression of differentiation markers was decreased in these cells during differentiation assays.
Conclusions: These preliminary results indicate that, besides β-E6s, α-E6 oncoproteins are also likely to inhibit the Notch signalling pathway by associating with MAML1, consequently downregulating cellular differentiation.

P16

SIRT7 Overexpression as a Prognostic Indicator in Triple-Negative Breast Cancer

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Keywords: Sirtuin7 Histone Deacetylases Triple Negative Breast Neoplasms

Background: Epigenetic regulation plays a key role in cancer by altering gene expression without changing the DNA sequence. Recent advances highlight chromatin-modifying enzymes as promising biomarkers and therapeutic targets. Among these, the sirtuin family has gained attention. Sirtuin 7 (SIRT7) is notable for its selective deacetylation of histone H3 at lysine 18 (H3K18ac), an epigenetic mark linked to transcriptional repression and oncogenic transformation. Although SIRT7 is involved in DNA repair, ribosome biogenesis, and metabolism, its clinical relevance in breast cancer subtypes remains unclear. Investigating its expression may inform new therapeutic strategies, particularly for subtypes lacking targeted treatments.
Patients and Methods: This study analysed SIRT7 expression in tumor tissues from 111 breast cancer patients, including 48 with triple-negative breast cancer (TNBC) and 63 with hormone receptor-positive, HER2-negative (ER+PR+HER2−) tumors. Total RNA was extracted, and SIRT7 expression was quantified via real-time qPCR, normalized to reference genes. Statistical analyses compared expression between subtypes and evaluated correlations with clinicopathological features. Kaplan–Meier analysis assessed the association between SIRT7 expression and overall survival.
Results: No significant difference in SIRT7 expression was found between the subtypes. However, in the TNBC group, SIRT7 overexpression was significantly associated with shorter overall survival ($p = 0.037$). The mean survival was 39 months for the high-expression group versus 52 months for the low-expression group. No survival correlation was observed in the ER+PR+HER2− group. Additionally, in TNBC, high SIRT7 expression was associated with tumors smaller than 2 cm.
Conclusions: SIRT7 overexpression correlates with worse survival in TNBC, suggesting its potential role as a prognostic biomarker and a candidate for future targeted therapies. These findings support further investigation into SIRT7's role in epigenetic-based treatment strategies, particularly for aggressive and treatment-resistant breast cancers like TNBC.

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SESSION 3

TUMOR MICROENVIRONMENT - FRIEND OF FOE

P17

Exploring the expression and prognostic utility of NISCH in primary and metastatic rectal cancer

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Keywords: liver metastasis, NISCH, prognostic biomarker, rectal cancer

Background: Rectal adenocarcinoma (READ) ranks 8th in terms of incidence and 10th in mortality rates, representing a significant public health concern worldwide. As a result, new diagnostic and prognostic biomarkers are urgently needed. NISCH has been identified as both a tumor suppressor and a positive prognostic marker in breast and ovarian cancers. Recent analyses of NISCH across various tumor types have revealed its context-dependent role. However, NISCH has not been extensively studied in READ. The aim of this study was to analyze NISCH expression in both primary and metastatic READ and evaluate its prognostic potential.
Material (Patients) and Methods: NISCH levels in normal and READ samples were obtained through Xena Functional Genomics Explorer, TIMER, and GEPIA2 online tools. Additionally NISCH was analysed by RT-qPCR in our two cohorts with paired samples: cohort 1 consisted of 28 patients with primary READ with adjacent normal rectal tissue, and the cohort 2 with 17 patients with rectal cancer with liver metastasis (RCLM) and adjacent normal liver tissue. GAPDH levels were used for normalization. Data were analysed using the 2-dCt method. Kaplan–Meier (KM) analysis was used to evaluate overall survival (OS) and disease-free survival (DFS) in cohort 1 and time to recurrence (TTR) in cohort 2 based on NISCH expression.
Results: In silico analyses using Xena and TIMER online tools revealed no significant difference in NISCH expression between READ and normal rectal tissue. In contrast, GEPIA2 analysis indicated that NISCH was reduced in READ. Due to these inconsistent findings across databases, we further evaluated NISCH expression on our cohorts. No significant difference in NISCH expression was observed between READ and adjacent normal rectal tissue in cohort 1. Also, there was no difference in NISCH expression between primary and metastatic READ from cohort 2. However, NISCH was significantly downregulated in RCLM compared to healthy liver tissue. KM analysis showed no association between NISCH expression and OS or DFS (cohort 1) or TTR (cohort 2).
Conclusions: While NISCH expression did not differ between primary READ, adjacent normal tissue, or metastatic lesions, it was significantly downregulated in liver metastases compared to healthy liver tissue, suggesting a potential role in the metastatic liver microenvironment. NISCH expression showed no prognostic value for OS, DFS or recurrence in the analyzed rectal cancer cohorts.

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P18

Analysis of NK-92 cytotoxicity against different colon cancer cell lines

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Keywords: NK cells, colon cancer, NKG2D, cytotoxicity

Background: Natural Killer (NK) cells play a vital role in the immune defense against colorectal cancer (CRC). Their direct cytotoxic activity is driven through activating NK cell receptors and their ligands on target cells, leading to tumor cell death by releasing lytic granules such as perforin. This study aimed to assess how different CRC cell lines, which reflect varying stages of tumor progression, genetic mutations, and immune evasion strategies, affect NK cytotoxicity, production of perforin and interferon-gamma (IFN-γ), as well as the expression of NKG2D and PD-1 receptors.
Materials and Methods: The cytotoxic activity of NK-92 cells, a commercially available NK cell line, was evaluated against six CRC cell lines (SW480, SW620, HT-29, HCT116, DLD-1, and Caco2). NK-92 cells were co-cultured with fluorescently pre-labeled target cells at varying effector-to-target (E:T) ratios, and target cell lysis was assessed using flow cytometry (FACS). Additionally, FACS analysis was used to examine the expression of NKG2D and PD-1 receptors, as well as the production of perforin and IFN-γ.
Results: At the highest E:T ratio tested (10:1), NK-92 cells exhibited enhanced cytotoxic activity against the SW480 CRC cell line and its metastatic derivative, SW620. Notably, co-cultivation with SW620 cells led to a marked downregulation of the activating receptor NKG2D and a reduction in perforin production in NK-92 cells, which corresponded with decreased cytotoxic efficacy compared to that observed with the primary SW480 cells. HT-29, HCT116, and DLD-1 demonstrated greater susceptibility to NK-92-mediated cytotoxicity than Caco-2 cells, the latter exhibiting less than 10% cell lysis. This may be attributed to the close resemblance of Caco-2 cells to normal enterocytes, characterized by the expression of epithelial ligands typically found in non-transformed tissue. Interestingly, following a 4-hour co-culture with NK-92 cells, HT-29, HCT116, and DLD-1 cells upregulated expression of the immune checkpoint ligand PD-L1, yet no changes in PD-1 expression were detected on NK-92 cells. Overall, NK-92 cells produced low levels of IFN-γ in response to all CRC cell lines.
Conclusions: NK-92 cell cytotoxicity varied across CRC cell lines. Reduced NKG2D expression and perforin production following co-culture with the metastatic SW620 suggest impaired NK-92 killing capacity. Additionally, the observed upregulation of PD-L1 on CRC cells might contribute to NK-92 dysfunction.

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P19

Proteomic analysis of endothelial cell derived secretomes exposed to hypoxia-induced pharyngeal cancer cell-derived exosomes

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Keywords: hypoxic exosome, metastasis, microenvironment

Background: Head and neck squamous cell carcinomas (HNSCCs), which are associated with high mortality rates, exhibit enhanced metastasis, invasion, angiogenesis, and immune evasion, despite advancements in diagnosis and treatment. Recent studies indicate that exosomes, key mediators of tumor–microenvironment communication, contribute to prognostic and pathological alterations in cancer. However, the composition of exosomes released under hypoxia in cancers such as pharyngeal cancer and their impact on the tumor microenvironment remain poorly understood. This study aimed to investigate the changes in the protein profiles of secretomes from endothelial cells exposed to hypoxia-

induced tumor-derived exosomes (hiTDExs) from pharyngeal cancer cells.
Material and Methods: Protein profile changes in human umbilical vein endothelial cells (HUVECs) treated with hiTDExs were analyzed using a human cytokine antibody array assay. The expression of the target proteins was examined in HNSCC patient tissues with and without metastasis.
Results: hiTDExs significantly modulated cytokine expression in HUVEC-derived secretomes. Array analysis revealed at least 10 cytokines with altered expression compared to normoxic exosome-treated controls. Notably, CCL26, EGFR, IL-3, and IL-8 expression was significantly altered. The functional role of these factors in remodeling the pharyngeal cancer microenvironment, particularly in promoting metastasis and hypoxia, requires further investigation.
Conclusions: Our findings offer novel insights into how dynamic interactions between endothelial cells and hiTDExs drive metastasis. While chemokine-based therapies remain under development, CCL26 emerges as a promising target for future HNSCC treatments.

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P20

Exosomes as Key Mediators of Fibroblast Reprogramming into CAFs via Oncogenic miRNA Transfer and Activation of Cancer-Related Pathways

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Keywords: exosomes, fibroblasts, miR-21, oral cancer, tumor microenvironment

Background: The tumor microenvironment is shaped by complex interactions between tumor cells and stromal cells, among which fibroblasts play a key role. Exosomes, as important mediators of intercellular communication, transfer regulatory molecules—including microRNAs—that can modulate the behavior of recipient cells. This study aimed to investigate the effects of exosomes derived from healthy, premalignant, and malignant oral epithelial cells on the expression of key oncogenic signaling pathways in fibroblasts.
Methods: Exosomes were isolated from cell lines of healthy epithelium (HaCaT), dysplastic cells (DOK), and oral squamous cell carcinoma (SCC15 and SCC25). Expression of miR-21 was analyzed in exosomes from each group, followed by treatment of normal fibroblasts with these exosomes for 24 hours. Total RNA was extracted from fibroblasts using the Trizol method, and expression of genes involved in carcinogenesis (PIK3CA, AKT, NOTCH1, HES1) was analyzed by qPCR. Fibroblasts without treatment served as the reference control for gene expression normalization.
Results: MiR-21 expression was significantly higher in exosomes derived from malignant cells compared to those from healthy and premalignant cells, while no significant difference was observed between healthy and dysplastic cells. Fibroblasts treated with cancer-derived exosomes showed a significant upregulation of PIK3CA($p=0.041$), AKT ($p<0.0001$), NOTCH1 ($p=0.0003$), and HES1 ($p<0.0001$) compared to the healthy exosome-treated group. Treatment with dysplastic cell-derived exosomes resulted in a significant increase in AKT ($p=0.04$) and HES1 ($p=0.01$) expression. No changes were observed in fibroblasts treated with exosomes from healthy cells.
Conclusions: Exosomes from malignant and premalignant epithelial cells induce activation of oncogenic signaling pathways in fibroblasts, with miR-21 potentially playing a key role in fibroblast reprogramming toward a pro-tumorigenic phenotype.

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P21

Role of Eicosanoid Receptor in Stroma – Cancer Communication

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Keywords: CAF, PUFA, Metabolism, HCC, Tumor Microenvironment

Background: The tumor microenvironment (TME) is a complex and dynamic system composed of multiple cell types, including immune cells, endothelial cells, and cancer-associated fibroblasts (CAFs), which interact with cancer cells to influence tumor growth, metastasis, and resistance to therapy. Among these, CAFs are a particularly abundant and active component in many solid tumors, yet their role in cancer progression remains underexplored compared to immune or vascular interactions.
Recent studies have highlighted that CAFs can influence tumor biology through the secretion of cytokines, remodeling of the extracellular matrix, and metabolic interactions. However, our understanding of the specific surface receptors and signaling mechanisms mediating CAF–cancer cell crosstalk is limited. In this study, we investigate a novel G-protein-coupled receptor (GPCR), EICOR1, and its ligand EICO1, as potential mediators of CAF-driven effects in hepatocellular carcinoma (HCC), a highly aggressive liver cancer with limited therapeutic options.
Materials and Methods: To identify CAF-specific receptors relevant in HCC, we performed an integrative analysis of public single-cell RNA sequencing (scRNAseq) and spatial transcriptomics datasets from HCC tumors. Differential expression analysis revealed a subset of GPCRs selectively enriched in CAFs, among which the eicosanoid receptor EICOR1 was highly and specifically expressed.
We validated the spatial expression of EICOR1 using RNAscope in situ hybridization and spatial transcriptomics on patient-derived HCC tissue samples. To study its functional relevance, we employed the chick embryo chorioallantoic membrane (CAM) assay by co-engrafting human CAFs with liver cancer cells, in the presence or absence of EICOR1 activation.
In vitro assays were conducted to evaluate downstream signaling pathways and kinase activity in fibroblasts. Metabolomic profiling was performed on matched primary and secondary liver tumor samples from patients to assess the abundance of EICO1, the natural ligand of EICOR1. Furthermore, fibroblast-conditioned media were subjected to proteomic analysis to identify secreted factors that may mediate anti-angiogenic effects.
Results: CAF-specific Expression of EICOR1: scRNAseq and spatial transcriptomics data revealed that EICOR1 expression is restricted to the stromal compartment of HCC tumors, specifically in CAF populations, with negligible expression in tumor epithelial or immune cells. RNAscope analysis confirmed this localization in multiple patient samples. Functional Impact on Angiogenesis: In the CAM assay, fibroblasts expressing EICOR1, when co-engrafted with liver cancer cells and stimulated with EICO1, displayed a marked reduction in tumor-induced neoangiogenesis compared to control groups. This reduction was quantifiable through imaging and vascular marker analysis.
Mechanistic Insights: Kinase profiling in vitro indicated that EICOR1 activation leads to downregulation of several pro-tumorigenic kinases in fibroblasts, suggesting a reprogramming of their functional state. Among the most significantly altered were pathways associated with cell motility, inflammation, and extracellular matrix remodeling. Metabolomic Correlations in Human Tumors: Analysis of matched tumor and adjacent non-tumor liver tissue from patients showed that levels of the natural ligand EICO1 were significantly lower in the tumor regions. This suggests either ligand depletion or altered synthesis in the tumor microenvironment. Importantly, high EICOR1 expression in patient tumor samples was associated with better overall survival, supporting its potential protective role. Secretome Profiling: Proteomic analysis of fibroblast-conditioned media following EICOR1 activation revealed increased secretion of known anti-angiogenic proteins, including tissue inhibitors of metalloproteinases (TIMPs). These findings provide a possible mechanistic explanation for the observed anti-angiogenic phenotype.
Conclusions: Our findings establish EICOR1 as a novel CAF-specific eicosanoid receptor involved in modulating angiogenesis and tumor–stroma interactions in HCC. The selective expression of EICOR1 in the tumor stroma, its anti-angiogenic function upon activation, and the correlation of its expression with patient survival suggest that this receptor–ligand axis may serve as both a biomarker and a therapeutic target.
We propose that EICO1–EICOR1 signaling suppresses the pro-tumorigenic function of CAFs and supports an anti-angiogenic phenotype. These insights pave the way for the development of new therapeutic strategies that aim to reprogram or target the CAF compartment in liver cancer. Specifically, small-molecule agonists or mimetics of EICO1 could offer a novel approach to disrupt the tumor-promoting CAF–cancer cell communication network.
Currently, we are engaged in the synthesis and preclinical evaluation of compounds targeting this newly identified

EICO1–EICOR1 axis. We believe that targeting stromal communication offers a promising avenue for improving treatment outcomes in HCC and potentially other cancers where CAFs play a central role.
Note: The precise identity and structure of EICOR1 and EICO1 will be disclosed during the meeting.

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SESSION 4

IMMUNOONCOLOGY

P22

Integrated Clinical, Molecular, Microbiome, and Radiomic Profiling to Improve Immunotherapy Response Prediction in Inoperable NSCLC

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Keywords: immune checkpoint inhibitors, NSCLC, PD-L1, biomarkers, gene expression, microbiome, radiomics, prognosis, machine learning

Background: Checkpoint inhibitors represent one of the most innovative and prominent types of anticancer therapeutics. Besides their exceptional breadth of usage in solid tumors and remarkable responses in select patients, the majority of individuals fail to derive meaningful benefit. Unfortunately, no ideal biomarker currently exists to predict which patients will respond. In non-small cell lung cancer (NSCLC), treatment decisions are typically guided by the level of PD-L1 expression, yet its predictive value remains limited. Therefore, development of more accurate and sensitive predictive systems for immunotherapy response remains a priority.
Materials (Patients) and Methods: We conducted a set of complementary studies in Saint Petersburg institutions aimed at improving immunotherapy patient selection in NSCLC. A retrospective clinical analysis of 415 patients with inoperable NSCLC was performed to identify prognostic factors using Cox regression modeling. A prospective biomarker study involving 146 NSCLC patients assessed the predictive value of a novel 10-gene expression signature (CEI), quantified via PCR in tumor tissue. Microbiome profiling and immune microenvironment characterization were carried out in 63 patients using 16S rRNA sequencing and immune gene expression analysis, with response defined as lack of progression within 6 months. Baseline radiomic features from CT scans of 220 patients were analyzed using a comprehensive machine learning ensemble framework to predict long-term benefit (24-month overall survival).
Results: The clinical study identified six statistically significant prognostic factors (including lymph node status, NLR, and sex), which allowed classification into prognostic subgroups; patients with favorable profiles had significantly improved survival with checkpoint inhibitors ($p=0.045$). The gene expression study confirmed that high CEI values were associated with improved immunotherapy efficacy, with a 6-month progression-free survival rate of approximately 75% versus 35% in the low CEI group ($p<0.05$). Microbiome diversity indices (Chao1, Shannon) and specific taxa abundance correlated with clinical benefit, although no single genus or phylum was universally predictive. The radiomics ensemble model, combining clinical and imaging features, achieved an AUC of 0.86 for predicting 24-month survival, outperforming models based on either data type alone.
Conclusions: Our multidisciplinary results emphasize the inadequacy of PD-L1.

P23

Design and functional validation of self-deliverable siRNA for highly efficient suppression of PD-1 expression

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Keywords: immunotherapy, PD-1, siRNA

Background: The unique ability of small interfering RNAs (siRNAs) to suppress the expression of target genes makes them a promising tool in oncology, offering effective and safe competition to both low-molecular-weight targeted drugs and monoclonal antibodies. The possibility of targeting siRNAs to specific genes, even in the presence of individual mutations, opens up new opportunities for personalized oncology. However, the main and still unresolved challenge regarding siRNA application is the difficulty of delivering them into target cells, which hinders their adoption into clinical practice. Therefore, the aim of this study was to develop a unique cholesterol-modified siRNA molecule to silence the gene encoding the immune checkpoint protein PD-1. **Materials and Methods:** A standard protocol was used for isolating T-lymphocytes from peripheral blood by density gradient centrifugation (e.g., using Ficoll-Paque), after which T-lymphocytes were isolated from the mononuclear fraction using immunomagnetic separation. The passenger strand of a unique siRNA designed through bioinformatic methods was conjugated to a cholesterol molecule via a TEG linker. **Results:** Flow cytometric analysis of standard-activated T-lymphocytes showed that CD3-positive cells comprised 95 ± 6% of the samples. Flow cytometry results also demonstrated that within 6 hours after adding antiPD-1 siRNA-FAM at a concentration of 2 µM into the culture medium, more than 80% of T-lymphocytes contained the FAM label. This percentage decreased over time, likely due to siRNA degradation followed by the release and/or fading of the fluorescent label. Additionally, changes in the mRNA levels of the PDCD1 gene in activated T-lymphocytes were evaluated by qPCR at 24 and 48 hours after the addition of antiPD-1 siRNA (2 µM) to the culture medium. Twenty-four hours after treatment, the level of PDCD1 mRNA decreased by more than 80%, and after 48 hours it remained suppressed by more than 60%. **Conclusions:** The obtained results demonstrate the high efficiency of the developed self-deliverable antiPD-1 siRNA. The transfection efficiency and gene expression suppression levels either match or exceed those reported previously for PD-1-targeting siRNAs in published studies.

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The impact of TGFB1 and IFNG gene expression on progression of metastatic melanoma patients undergoing anti-PD-1 therapy

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Keywords: Interferon-gamma, Melanoma, Pembrolizumab, Transforming Growth Factor beta 1

Background: PD-1 checkpoint inhibitors like Pembrolizumab have improved outcomes in patients with metastatic melanoma (MM), but some patients still have disease progression, indicating the need for biomarkers of treatment response. Cytokines like transforming growth factor beta-1 (TGFB1) and interferon-gamma (IFNG) are implicated in immune regulation and tumor progression. Their predictive role in immunotherapy deserves further exploration.

Material and Methods: Twenty-one BRAF wild type MM patients were included in the study. TGFB1 and IFNG gene expression was quantified using TaqMan qPCR after total RNA isolation from peripheral blood mononuclear cells. Fold changes (FC) were calculated using the ddCt method with GAPDH as the reference gene. Expression was measured at baseline and before the 5th, 9th, 13th, and 17th cycles of immunotherapy. Patients were classified into the progression group (PG) (n=10) or non-progression group (NPG) (n=11) based on outcomes within one year. Baseline levels (BL) of TGFB1, IFNG, LDH, CRP, neutrophils (NEU), lymphocytes (LY), and NEU/LY ratio (N/L), were compared between groups using the Mann-Whitney test. Time to progression (TTP) was analyzed with Kaplan–Meier survival methods, and group differences were tested with the log-rank test. In TTP analysis, patients were divided into low or high expression group using median FC value of TGFB1 and IFNG. In NPG, longitudinal changes in cytokine gene expression were assessed with the Friedman test. In PG, TGFB1 and IFNG values at BL were compared with values at the time of progression using the Mann-Whitney test. Correlations between LDH, CRP, NEU, LY, N/L and TGFB1 and IFNG were tested with Spearman's rank correlation.

Results: There were no significant differences in TGFB1, IFNG, LDH, CRP, NEU, LY, N/L at BL between PG and NPG, but TGFB1 showed a trend toward lower values in PG (p=0.053). Log-rank analysis showed no significant link between cytokine gene expression at BL and disease progression, but lower values of TGFB1 trended toward shorter TTP (p=0.090). There was no correlation between LDH, CRP, NEU, LY, N/L and TGFB1 or IFNG expression. No significant differences existed in TGFB1 or IFNG levels over time in NPG, nor between BL and levels at the time of progression in PG.

Conclusions: In this cohort of MM patients treated with Pembrolizumab, lower BL TGFB1 indicated a trend towards shorter TTP and higher probability of progression. These findings suggest the predictive potential of TGFB1 that requires larger studies

SESSION 5

EXPLORING THE ROLE OF MDR MECHANISMS AND STEM CELL BIOLOGY IN CANCER AND CANCER TREATMENT STRATEGIES

P25

Expression profiling of p53 family isoforms in targeted therapy-resistant primary melanoma cell lines

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Keywords: alternative splicing, drug resistance, melanoma, MITF transcription factor, tumor suppressor protein p53

Background: Metastatic melanoma is the most aggressive form of skin cancer that is responsible for more than 80% of cutaneous cancer-related deaths. This cancer is characterized by a high frequency of somatic mutations, predominantly in the BRAF gene. Patients harboring the BRAF V600E mutation are treated with targeted therapies, including BRAF kinase inhibitors (BRAFi) alone or combined with MEK inhibitors (MEKi). Despite initial responses, resistance to these therapies develops rapidly, often driven by molecular alterations. One such alteration is the changed expression of p53 family isoforms, which regulate the activity of the tumor suppressor p53 and may influence resistance mechanisms.
Material and Methods: To better understand these molecular changes, we established and validated primary melanoma cell lines derived from metastatic melanoma patients who received combined BRAFi/MEKi therapy. We aimed to characterize the expression profiles of p53 family members in these cells. We assessed the expression of p53, p63, and p73 isoforms at both the mRNA and protein levels using quantitative PCR (qPCR) and Western blot analysis across various melanoma sublines. Additionally, we measured the expression of MITF, which encodes the central transcription factor that regulates melanogenesis, via qPCR. Expression of MITF in melanoma sublines was compared to that in non-melanoma cancer cell lines, including prostate, ovarian, and neuroblastoma lines.
Results: We observed that primary cell sublines differ in morphology and consist of three distinct subtypes: melanocytic cells, mesenchymal cells and a mixed population. In all cell sublines the expression of MITF gene was determined and compared with MITF expression in different non-melanocytic cancer cell lines. All melanoma sublines demonstrated higher MITF expression than the non-melanoma cancer lines. However, levels of MITF varied significantly among the melanoma sublines. The results of p53 isoform expression revealed the differences in both mRNA and protein expression levels between the individual cell sublines.
Conclusions: These preliminary findings suggest that specific p53 family isoforms may be associated with the development of resistance to combined BRAFi/MEKi targeted therapy in melanoma. Further research is required to confirm whether these isoforms can serve as reliable biomarkers for acquired therapy resistance and to explore their potential roles in treatment outcomes.

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SESSION 6

CANCER EPIDEMIOLOGY- STRATEGIES FOR PREVENTION AND EARLY DETECTION

P26

Glutathione-S transferase omega 1 and omega 2 gene polymorphisms modify susceptibility to brain tumor development

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Keywords: brain tumor, glutathione S-transferase omega, gene polymorphism

Background: Glutathione S-transferases (GSTs) play critical roles in detoxification, redox homeostasis, and modulation of cellular signaling pathways, thus participating in tumorigenesis through effects on cell survival, proliferation, and drug resistance. Among them, the omega-class of GSTs (GSTO1 and GSTO2) exhibit distinct enzymatic functions, including thioltransferase and dehydroascorbate reductase activity, contributing to the regulation of redox homeostasis and protein function. Given the central role of reactive oxygen species in brain tumor pathogenesis, and the fact that GSTO genes are highly polymorphic, investigating their variants may provide insight into individual susceptibility to brain tumor development.
Material and methods: GSTO1 rs4925 and GSTO2 rs156697 gene polymorphisms were determined by the qPCR method in 111 patients with brain tumors (classified into gliomas, meningiomas and other tumor types including pituitary adenomas and less common intracranial neoplasms) and 140 age- and gender- matched healthy controls.
Results: Homozygous carriers of the GSTO1 variant *A allele had a significantly reduced overall risk of brain tumor development compared to carriers of the referent genotype (OR=0.26, 95%CI:0.08-0.86, p=0.027). Subgroup analysis revealed that the observed significance was driven by the association between GSTO1 polymorphism and the risk of developing pituitary adenomas and less common intracranial neoplasms (OR=0.25, 95%CI:0.09-0.67, p=0.013), while no statistically significant associations were found for gliomas or meningiomas. The additional haplotype analysis confirmed that individuals with the GSTO H2 haplotype, defined as the concomitant presence of the variant GSTO1*A and GSTO2*G alleles, had decreased risk of developing pituitary adenomas and less common intracranial neoplasms (OR=0.13, 95%CI:0.02-0.74, p=0.023). Conversely, individuals with H3 haplotype including referent GSTO1*C and variant GSTO2*G alleles were at significantly higher risk for glioma (OR=2.94, 95%CI:1.21-7.15, p=0.018) and meningioma (OR=4.08, 95%CI:1.19-13.94, p=0.026).
Conclusion: Our results suggest that GSTO locus variants may influence susceptibility to brain tumors depending on the histological subtype. These findings support further investigation into GSTO variants as potential genetic markers for brain tumor risk stratification.

P27

ABCB1 polymorphisms rs2032582 and rs1045642 in epithelial ovarian cancer pathogenesis and progression

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Keywords: ABCB1, epithelial ovarian cancer, polymorphism

Background: The ABCB1 gene encodes for ATP-binding cassette subfamily B member 1 (ABCB1) P-glycoprotein (Pgp). It is a transmembrane efflux pump for a broad range of xenobiotics including carcinogenic substances as well as anticancer drugs. ABCB1 polymorphisms rs2032582 (A>C/T in exon 21) and rs1045642 (A>G in exon 26) could change Pgp expression and cellular clearance of xenobiotics, and thus influence cancer development and clinical outcome. The effect of these polymorphisms on epithelial ovarian cancer (EOC) onset and progression is still undefined.
Patients and Methods: The epithelial ovarian tumors (75 malignant and 24 benign) were genotyped by TaqMan allelic discrimination real-time PCR. The association of genotypes with EOC development was evaluated by $\chi 2$ test and risk ratio (RR) with 95% confidence interval (CI). The relationship of genotypes and haplotypes with clinicopathological (CP) characteristics of EOC was analyzed by logistic regression. Fifty-eight patients who received taxane/carboplatine chemotherapy were followed-up for progression-free survival (PFS). PFS between genotypes was compared by the log-rank test.
Results: The rs2032582 CC genotype was significantly more frequent in malignant vs. benign tumors (43.5% vs. 9.1%) compared to AA plus AC (p=0.007, RR (95%CI): 4.8 (1.2-18.4)). Also, the rs1045642 GG genotype was significantly more frequent in malignant vs. benign tumors (36.5% vs. 12.5%) compared to AA plus AG (p=0.05, RR (95%CI): 2.9 (1.0-8.8)). The investigated genotypes and haplotypes did not correlate with EOC CP features. In the first five years of follow-up, the AA vs. AC plus CC rs2032582 genotypes showed a trend toward lower PFS (median 12.3 vs. 22.2 months; p=0.076). Regarding AA vs. AG plus GG rs1045642 genotypes, the difference in PFS was significant (median 10.2 vs. 26.4 months; p<0.001).
Conclusions: The results indicate that ABCB1 rs2032582 CC and rs1045642 GG genotypes may be risk factors for EOC development. Furthermore, the rs2032582 AA and rs1045642 AA genotypes could be biomarkers of disease progression. Larger cohorts are needed to confirm these findings.

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Distribution of EGFR -216G/T (rs712829) polymorphism: Implications for cancer risk and pharmacogenomics

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Keywords: EGFR; non-small-cell lung cancer; -216G/T polymorphism; rs712829; SNP

Background: The epidermal growth factor receptor (EGFR) gene polymorphism rs712829 has been associated with non-small cell cancer (NSCLC) risk and drug response, with variability across populations. This study aimed to investigate the genotype and allele distributions of rs712829 across East Asian (EAS), European (EUR), and Serbian (SRB) populations, including an analysis of gender-based differences.

Materials and Methods: We analyzed genotype data from the 1000 Genomes Project (EAS and EUR populations) and a cohort of healthy Serbian volunteers from our previous study, focusing on the rs712829 SNP. Genotype and allele frequencies were calculated for each population, stratified by gender. Fisher's exact test or chi-squared test was applied to assess gender-based differences in genotype and allele distributions. All analyses were performed using R version 4.3.3 (R Core Team, 2024) with the following packages: dplyr, tidyr, ggplot2, ggpubr, readr, and stringr.
Results: In the EAS population, the G/G genotype predominated in both females (87.7%) and males (89.8%), with low frequencies of G/T (11.9% and 9.8%, respectively) and T/T (≤0.41%). In contrast, the EUR population showed more variability: in females, G/G occurred in 46.8%, G/T in 45.2%, and T/T in 8.0%; in males, G/G was 49.6%, G/T in 37.9%, and T/T in 12.5%. Allele G was more frequent in EAS (F: 93.6%, M: 94.7%) than in EUR (F: 69.4%, M: 68.5%). Gender-based comparisons in both EAS and EUR populations showed highly significant differences in genotype and allele distributions (Fisher's exact and chi-square tests, all p-values < 0.01). In the cohort from SRB population (n=40), genotype frequencies were G/G: 47.5%, G/T: 42.5%, and T/T: 10%. Allele G frequency was 68.8%, and allele T was 31.2%. No statistically significant differences by gender were observed in the Serbian population for either genotype (p = 0.8) or allele frequencies (p=0.63).
Conclusions: Genotype and allele distributions of EGFR rs712829 significantly differ between East Asian and European populations, with the EAS group exhibiting a much higher prevalence of the G allele. The Serbian population showed a distribution more similar to EUR, with no significant gender-based differences. These findings underline population-specific genetic variability and emphasize the importance of considering ethnicity and sex in pharmacogenetic and epidemiological research involving EGFR variants.

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P29

The role of glutathione S-transferase omega 1 and omega 2 polymorphisms in susceptibility and prognosis of colorectal cancer

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Keywords: colorectal cancer; GSTO; polymorphism, risk, prognosis

Background: Despite advancements in screening programs, colorectal cancer (CRC) is still frequently diagnosed at advanced stages. The glutathione S-transferase (GST) family of enzymes plays a key role in the detoxification of xenobiotics. Among them, the omega class (GSTO) is particularly important for maintaining redox homeostasis, regulating pro-inflammatory signaling, and modulating apoptosis and cell survival pathways. The aim of this study was to evaluate the possible role of gene polymorphisms of the GSTO1 and GSTO2 in the risk and prognosis of CRC.
Materials and Methods: The study included 140 patients with a histopathologically confirmed diagnosis of CRC and 140 age and gender-matched controls. Genomic DNA was extracted from peripheral blood using the QIAamp DNA Blood Mini Kit. GSTO1 (rs4925) and GSTO2 (rs156697) polymorphisms were determined using the real time PCR amplification method. The median follow-up of patients was 44.00 (IQR 7.5) months.
Results: The obtained data showed thatcarriers of at least one GSTO1*A variant allele had a six-fold decreased CRC risk compared to those carrying the referent GSTO1*CC genotype (OR=0.16, 95%CI=0.09–0.27, p<0.001). In the case of the GSTO2 polymorphism, individuals with at least one variant GSTO2*G allele had a significantly decreased CRC risk (OR=0.59, 95%CI=0.36–0.95, p=0.029) compared to individuals with the referent GSTO2*AA genotype. Additionally, individuals carrying a combination of one variant allele of both GSTO1 and GSTO2 had a significantly lower CRC risk (OR=0.30, 95%CI=0.18–0.50, p<0.001). These findings were confirmed by a haplotype analysis; the individuals with the H3 haplotype, represented by one copy of the variant GSTO1 (*A) and one copy of the variant GSTO2 (*G) alleles had the lowest CRC risk (OR=0.42, 95%CI=0.25-0.70, p=0.001). No statistically significant effect of either GSTO1 or GSTO2

polymorphisms was observed in terms of overall survival in CRC patients (p>0.05).
Conclusions: Our results suggest that GSTO1 and GSTO2 polymorphisms may confer CRC risk.

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A Case of Hereditary Hemorrhagic Telangiectasis

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Keywords: Bevacizumab, Bleeding, Hereditary hemorrhagic telangiectasia

Background: Hereditary hemorrhagic telangiectasia (HHT, also known as Osler-Weber-Rendu disease) is a multisystemic vascular disorder characterized by abnormal angiogenesis and inherited in an autosomal dominant manner. HHT is the second most common hereditary bleeding disorder worldwide. It affects approximately 1 in 5,000 individuals, or an estimated 1.4 million people globally, making it twice as prevalent as hemophilia A and 12 times more common than hemophilia B. HHT is characterized by vascular malformations in the nasal mucosa, skin, gastrointestinal tract, brain, lungs, and liver, and leads to telangiectasia in the nasal mucosa, resulting in recurrent epistaxis. This condition is observed in approximately 95% of HHT patients, with the average age of onset around 12 years and an epistaxis frequency of approximately 18 episodes per month.
Patient: A 76-year-old male patient has experienced epistaxis since the age of 10 and oral bleeding for the past 4 years. The patient underwent inguinal hernia surgery in 1995 and a gastric biopsy in 2023, during which no hemostatic complications were observed. He has a history of enoxaparin and warfarin (Coumadin) use due to atrial fibrillation. The patient has recurrent episodes of epistaxis and oral bleeding approximately every two weeks and a history of frequent erythrocyte transfusions. His hemoglobin levels have remained around 7 mg/dL. On December 8, 2023, bevacizumab therapy (anti-VEGF monoclonal antibody) was initiated at a dose of 5 mg/kg every two weeks. By the first month of treatment, his hemoglobin level had increased to 10 mg/dL, and by the second month, to 12 mg/dL. When bevacizumab therapy was temporarily discontinued, bleeding episodes recurred; therefore, it was decided to continue maintenance therapy at intervals of once every three weeks.
Conclusion: This case demonstrates that epistaxis, one of the hallmark symptoms of HHT, can begin in childhood and persist into advanced age. Following bevacizumab therapy, a significant improvement in hemoglobin levels was achieved; however, bleeding recurred upon discontinuation of the treatment. This finding supports the potential efficacy of anti-VEGF therapy in managing HHT-related bleeding. Furthermore, treatment planning should be carried out with caution, considering comorbid conditions such as the use of anticoagulants.

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XRCC1 1196A>G and RAD51 135G>C polymorphic variants in rectal cancer: significance for cancer risk and response to chemoradiotherapy

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Keywords: Chemoradiotherapy, Polymorphism, single nucleotide, RAD51 recombinase, Rectal neoplasms, X-ray repair cross complementing protein 1

Background: X-ray repair cross-complementing protein 1 (XRCC1) and DNA repair protein RAD51 homolog 1 (Rad51)

are proteins involved in the repair of DNA damage. XRCC1 participates in base excision repair of DNA single-strand breaks caused by ionizing radiation and alkylating agents, while Rad51 plays a crucial role in the repair of double-strand breaks through homologous recombination. The aim of this study was to analyze the association of XRCC1 1196A>G and RAD51 135G>C single nucleotide polymorphisms (SNPs) with the risk of rectal cancer occurrence in Serbia, as well as their impact on the response to preoperative chemoradiotherapy.
Material and Methods: For XRCC1 SNP analysis, a total of 48 patients with locally advanced rectal cancer and 71 healthy controls were included in this case–control study. For RAD51 SNP analysis, 43 patients and 71 healthy controls were analyzed. Restriction fragment length polymorphism analysis was used for genotyping. Statistical significance was set at $p < 0.05$.
Results: The distribution of XRCC1 1196 A>G and RAD51 135G>C genotypes in patients and controls did not deviate from Hardy–Weinberg equilibrium. Using both dominant and recessive models, the XRCC1 was not found to be associated with an increased risk of rectal cancer. Examination of the association of polymorphisms in the XRCC1 Gln399Arg gene with the response to therapy showed that the presence of the marker allele A carries a risk for a poor therapeutic outcome (OR: 0.26, 95% CI: 0.07–0.99, $p = 0.044$). Analysis of RAD51 135G>C polymorphism showed no statistically significant association with the risk of rectal cancer or response to chemoradiotherapy. However, in male patients, the GG genotype showed an increased cancer risk (OR: 3.72, 95% CI: 1.34–10.36, $p = 0.009$).
Conclusions: Although a statistically significant association of RAD51 135G>C with disease risk in the male population was demonstrated, a deviation from Hardy–Weinberg equilibrium was noted. This may be due to the small sample size, thus these preliminary findings need to be validated using a larger case–control study. This study might be useful for future meta-analyses and construction of multi-gene, population-specific cancer risk prediction panels.

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The clinical significance of ATG5, ATG10 and ATG16L1 genes polymorphisms in advanced melanoma

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Keywords: Autophagy, Gene polymorphism, Melanoma, Survival

Background: Autophagy is a highly conserved catabolic process involved in maintaining cellular homeostasis. The role of autophagy in tumors (including melanoma) is still controversial since it differs during malignant transformation/ progression. Of note, autophagy is often increased in BRAF-positive melanomas where it impairs the response to targeted therapy. In this study, we analyzed six polymorphisms in autophagy-related genes (ATG16L1, ATG5 and ATG10) regarding their impact on clinicopathological characteristics and melanoma course.
Patients and Methods: The total of 149 patients with advanced melanoma were genotyped for rs2241880, rs2245214, rs510432, rs1864182, rs1864183 and rs1051423. Genotyping included DNA isolation from peripheral blood and allelic discrimination using TaqMan genotyping SNP assays for the StepOne Plus Real Time PCR System.
Results: Frequencies of analyzed genotypes were similar to those reported for other European populations. No association between ATG genotypes and clinical/pathologic characteristics was observed. However, ATG10 rs1864182 was associated with baseline (BL) absolute neutrophil count (ANC) (CC vs. AA; p=0.047), absolute platelet count (APC) (C vs. AA; p=0.042), neutrophils-to-lymphocytes ratio (CC vs. A; p=0.026), monocytes-to-lymphocytes ratio (CC vs. A; p=0.05), neutrophils x platelets x monocytes – to lymphocytes ratio (NPM/L) (CC vs. AA; p=0.016) and neutrophils x platelets – to lymphocytes ratio (NP/L) (CC vs. AA; p=0.045), respectively. The ATG10 rs1864183 was associated with BL ANC (CC vs. G; p=0.082), absolute monocyte count (CC vs. TT; p=0.04), APC (C vs. TT; p=0.014), NPM/L (CC vs. TT; p=0.007) and NP/L (CC vs. TT; p=0.055), respectively. In addition, patients with at least one rs2245214 C allele had better progression free survival (PFS) (p=0.029), distant metastasis free survival (DMFS) (p=0.012) and overall survival (p=0.017), respectively. In BRAF-negative patients, the presence of rs1051423 T allele was associated with better PFS (p=0.012), while patients with at least one rs1864182 allele had superior DMFS.

Conclusions: Variations in ATG10 and ATG5 genes are associated with blood/inflammatory cells counts/ratios at BL and clinical course of advanced melanoma.

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Identification and characterization of microRNAs involved in the progression from normal oral mucosa to oral cancer

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Keywords: oral mucosa, oral leukoplakia, oral cancer, miRNA

Background: The development of oral cancer is a complex, multistep process involving the transformation of normal oral mucosa into premalignant lesions, such as oral leukoplakia with dysplasia, and eventually into oral cancer. A key feature of this transformation is the deregulation of small non-coding RNA molecules, microRNAs (miRNAs). The identification of miRNAs that are consistently deregulated at different stages of oral carcinogenesis is crucial to gaining deeper insights into the molecular changes involved. Our aim was to identify and characterize commonly deregulated miRNAs in normal oral mucosa, oral leukoplakia, and oral cancer using publicly available databases.
Material and Methods: The National Center for Biotechnology Information Gene Expression Omnibus (NCBI GEO) was utilized to identify databases containing miRNA expression data from normal oral mucosa, oral leukoplakia, and oral cancer. Data from the GSE246050 dataset were analyzed using GEO2R software. Raw fastq files were retrieved from Sequence Read Archive (SRP468035). HISAT2 and StringTie were used for identification and quantification of miRNA transcripts. A list of miRNAs commonly deregulated was further characterised by miRNet v2.0 software.
Results: In total 112 genes were identified as commonly differentially deregulated across normal oral mucosa, leukoplakia with dysplasia and oral cancer. Among them were 6 microRNA genes: MIR429, MIR6726, MIR135B, MIR204, MIR139 and MIR211. Analysis of expression profiles of mature transcripts of these miRNA genes, revealed significant changes in expression of hsa-miR-429, hsa-miR-135b-5p, hsa-miR-135b-3p, hsa-miR-204-5p, hsa-miR-139-5p, hsa-miR-139-3p and hsa-miR-211-5p. We observed progressive upregulation of hsa-miR-429 and hsa-miR-135b-5p/3p, while downregulation of hsa-miR-204-5p, hsa-miR-139-5p/3p and hsa-miR-211-5p in progression from normal oral mucosa to oral cancer. To investigate the function of all genes regulated by miRNAs, a hypergeometric test and the KEGG database were used. Pathways in cancer were significantly enriched with the largest number of genes.
Conclusions: Progression from normal oral mucosa to oral cancer might be characterized by up-regulation of hsa-miR-429, hsa-miR-135b-5p/3p and down-regulation of hsa-miR-204-5p, hsa-miR-139-5p/3p and hsa-miR-211-5p. These results are good starting point for further validation of miRNA candidates in oral carcinogenesis in a larger group of clinical samples.

Acknowledgments and funding: This study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia [grant agreement: 451-03-137/2025-03/200178].

SESSION 7

ADVANCEMENTS IN MOLECULAR DIAGNOSTICS AND CANCER BIOMARKERS IN ONCOLOGY

P34

Beyond coding sequences: the role of the long non-coding RNA NBAT1 in HPV-positive cancer samples and cell lines

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Keywords: HPV, lncRNA, oncoproteins

Backround: Long non-coding RNAs (lncRNAs) are RNA transcripts longer than 200 nucleotides that, despite lacking coding potential, regulate diverse biological processes, including epigenetic modifications, transcription, and translation. The human genome contains approximately 15,000 lncRNA genes, with most of their functions still unknown. Recent studies have highlighted their involvement in pathological states, particularly in cancer. HPV oncoproteins E6 and E7 are known to alter the expression of over 1,400 lncRNAs, although the biological significance of many of them remains unclear. This study aimed to investigate the role of selected lncRNAs in HPV-associated head and neck malignancies, comparing them with cervical samples to determine whether HPV's effects are site-specific or universal.
Materials and methods: Based on literature and database searches, lncRNAs interacting with proteins implicated in HPV-driven carcinogenesis (MMP7, CDH1, GLI1, and KRT18) were identified, leading to the selection of five lncRNAs (FEZF1-AS1, FUNDC2P4, GAS5, FOXCUT, and NBAT1) for further analysis. Total RNA was extracted from tumor samples and cell lines and analyzed via RT-PCR.
Results: NBAT1 emerged as the most promising candidate, showing increased expression with higher tumor grade in head and neck cancers and significantly lower expression in HPV-positive tumors ($p = 0.05$). Due to the limited number of cervical lesion samples, no clear trend was observed in that group. Additional analyses in HPV+ and HPV- cell lines revealed no statistically significant differences in NBAT1 expression, although HPV- cervical cancer cells (C33A) showed a trend toward higher expression. A plasmid encoding NBAT1 was used to transfect various cell lines, and functional assays indicated that NBAT1 overexpression affected cell proliferation and migration. Finally, neither silencing of HPV16 and HPV18 E6/E7 in HeLa and CaSki cells nor overexpression of these oncoproteins in C33A cells significantly altered NBAT1 levels, suggesting that its expression is regulated by alternative mechanisms.
Conclusions: These findings position NBAT1 as a potential biomarker in HPV-related head and neck cancers and support further investigation into its role in HPV-independent tumor progression.

Acknowledgments and funding: This project was funded through the European Union Next generation plan.

P35

Diagnostic potential of SMAD7 and SMAD4 expression and their ratio in locally advanced rectal cancer and colorectal liver metastasis

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Keywords: Colorectal Cancer, Metastasis, Rectal Cancer, SMAD7, SMAD4

Background: Given the important role of SMAD signaling in mediating the TGF-β pathway activity in cancer, and the critical functions of both the inhibitory SMAD7 and the common mediator SMAD4 in this process, this study investigated the diagnostic relevance of the mRNA expression levels of these two genes and their ratio in locally advanced rectal cancer (LARC) and colorectal liver metastases (CLM).
Patients and Methods: Relative expression levels of SMAD7 and SMAD4 were measured in tumor and corresponding non-tumor tissues from 19 LARC and 16 CLM patients using quantitative real-time PCR (qPCR) and normalized to the housekeeping gene GAPDH. To evaluate the potential of the investigated gene expression levels and their ratio in distinguishing tumor from matched non-tumor tissues in LARC and CLM, Receiver Operating Characteristic (ROC) analysis was performed, and their translational value as diagnostic biomarkers was assessed based on the area under the curve (AUC), 95% confidence intervals (CI), and p-values. AUC values below 0.6 were considered poor, between 0.6 and 0.7 fair, 0.7 to 0.8 good, 0.8 to 0.9 very good, and above 0.9 excellent for the discriminatory power of the biomarker. Optimal cut-off values for differentiating tumor from non-tumor tissue were determined for each expression pattern using the maximum value of Youden's index (Youden's index = sensitivity + specificity – 1). Expression patterns were considered useful biomarkers if AUC>0.600 and p<0.05.
Results: In LARC patients, SMAD4 gene expression and the SMAD7/SMAD4 ratio demonstrated good diagnostic potential for distinguishing tumor from matched non-tumor tissue (AUC=0.731, p=0.015 and AUC=0.745, p=0.010, respectively), whereas SMAD7 expression alone did not perform well as a diagnostic biomarker (AUC=0.593, p=0.328). In CLM patients, SMAD7 and SMAD4 expression showed good to excellent diagnostic performance in distinguishing metastatic from matched non-tumor liver tissue (AUC=0.719, p=0.035 and AUC=0.973, p<0.0001, respectively), while the SMAD7/SMAD4 ratio did not prove to be a reliable diagnostic biomarker (AUC=0.629, p=0.214).
Conclusions: SMAD4 expression and the SMAD7/SMAD4 ratio showed promising diagnostic potential in LARC, while both SMAD7 and SMAD4 expression demonstrated strong discriminatory power in CLM, underscoring the need for validation in larger, independent patient cohorts.

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P36

ALDOA-212 noncoding transcript – a potential biomarker for colorectal cancer

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Keywords: biomarker, colorectal cancer, noncoding RNA

Background: Colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer-related

deaths worldwide. Aldolase A (ALDOA) has been linked to CRC progression by influencing cell proliferation and metastasis. Recently, noncoding transcripts of protein-coding genes have been reported as promising diagnostic and prognostic CRC biomarkers. The aim of this study was to obtain a transcriptional profile of ALDOA-212 (ENST00000566130.1), a noncoding transcript with a retained intron, in malignant and non-malignant cell lines and their subcellular compartments (nucleus, cytoplasm and exosomes), as well as in patients' tumorous and healthy epithelial tissues.
Patients and Methods: Malignant HCT116, DLD-1, and SW620 and non-malignant human colon epithelial cell line HCEC-1CT were cultivated in 3D as spheroids for 7 days. Tumorous tissues and samples of healthy mucosa from 5 CRC patients were collected at the Clinic for Digestive Surgery, University Clinical Centre of Serbia. Total and compartmental RNA was extracted using commercial kits. RNA sequencing was performed on ribosomal-depleted total RNA from cell lines using Illumina's NovaSeq6000 platform. Patients' total RNA and compartmental RNA from cell lines were analyzed by qPCR. ALDOA-212 expression levels were also examined using available human databases via UCSC Xena Functional Genomics Explorer, comparing primary tumor (TGCA Colon Adenocarcinoma database) with normal tissue (GTEx Colon database; total of 596 samples), as well as with solid normal tissue (TGCA Solid Tissue Normal database; total of 329 samples). Additionally, AnnoLnc2 and lncLocator tools were used to assess the potential subcellular localization.
Results and Conclusions: ALDOA-212 was upregulated in all three malignant cell lines compared to non-malignant cell line, while it was marginally downregulated in patient's tumorous tissue in comparison to healthy mucosa (p=0.06). Comparisons among larger available datasets showed no statistically significant difference between malignant and normal tissues. ALDOA-212 was predicted to be localized in the nucleus or cytosol. However, we detected this transcript in all examined compartments in HCEC-1CT and HCT116 cell lines. In DLD-1, it was detected in the nucleus and exosomes, while in SW620, it was found exclusively in exosomes, suggesting its metastatic potential. CRC diagnostic and prognostic significance of ALDOA-212 requires further confirmation and functional characterization.

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P37

Analysis of Variants in Cytochrome P450 Superfamily Genes as Predictive Markers for Neoadjuvant Chemoradiotherapy in Rectal Cancer

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Keywords: biomarkers, genes, rectal cancer, SNPs

Background: Patients with locally advanced rectal cancer (LARC) often receive neoadjuvant chemoradiotherapy (nCRT) based on 5-fluorouracil (5-FU). Cytochrome P450 (CYP) enzymes, involved in drug metabolism and carcinogenesis, may influence response to therapy. Variability in CYP gene expression makes them promising biomarkers for predicting treatment outcomes in rectal cancer.
Material and methods: Genomic DNA from 38 LARC patients treated with standard nCRT (fluorouracil + leucovorin + radiotherapy) was analyzed. Clinical response was assessed by tumor regression grade (TRG). Seven patients with extreme responses – good (3 TRG1 and 2 TRG2) and poor (2 TRG5) were selected for whole-exome sequencing (WES). Candidate predictive variants were identified based on their differential presence between responder and non-responder groups and biological relevance (localization in coding region or regulatory elements and enzyme-altering effects). Selected variants were validated in 29 moderate-response patients (15 TRG3 and 14 TRG4) by targeted sequencing.
Results: Two genetic variants were selected according to the criteria outlined above: rs149012039, which is located within the CYP2D7 pseudogene and represents a frameshift variant, and rs3093200, which is located in the first exon of the CYP4F2 gene and has a damaging effect on the protein. Validation in the remaining samples showed that neither variant was present in patients with a moderate response to therapy.
Conclusions: The presence of variants rs149012039 and rs3093200 in individuals with a good clinical response and their absence in individuals with a moderate or poor response supports their potential as predictive biological markers for response to nCRT in rectal cancer.

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P38

GPX1 gene polymorphism (rs1050450) affects overall survival of patients with prostate cancer: Serbian cohort study

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Keywords: glutathione peroxidase 1, gene polymorphism, prostate cancer, survival

Background: It is well established that redox homeostasis plays an important role in both the development and progression of cancer. Glutathione peroxidase 1 (GPX1) seems to be one of the main regulators of intracellular hydrogen peroxide (H_2O_2) levels. By altering its enzymatic activity, the GPX1 gene polymorphism might affect H_2O_2 concentration, thus, alternating signaling pathways involved in apoptosis and cell proliferation. The aim of this study was to evaluate the possible prognostic role of GPX1 rs1050450 gene polymorphism in patients with prostate cancer (PC).
Material and Methods: Total of 235 Serbian patients aged 68.84 ± 6.95 with pathohistologically confirmed prostate cancer were included in this study. DNA was isolated from whole blood and GXP1 rs1050450 gene polymorphism was determined by real-time polymerase chain reaction (PCR). To address the possible prognostic role of this polymorphism, patients' survival outcomes were followed from 2014-2022. For the analysis of the overall survival, Kaplan-Meier curves were generated. Two distinctive models were composed to inspect not only the independent, but also the combined effect of this gene polymorphism with known prognostic risk factors to evaluate the hazard ratio (HR) for the fatal outcome.
Results: Most patients had prostate-specific antigen (PSA) over 20 ng/mL at the time of diagnosis and Gleason score 6. During the follow-up period of eight years, 13 (5%) patients were lost. Patients with GPX1*ProPro genotype, indicating those with higher enzymatic activity, lived significantly shorter than those carrying at least one GPX1*Leu allele, indicating lower enzymatic activity (p = 0.031). When analyzed independently, GPX1*ProPro genotype conferred 1.6 times higher risk for fatal outcome (HR = 1.65; 95% CI = 1.04 – 2.60; p = 0.034). However, when Gleason score and PSA were included into the analysis, this risk increased to ~2.5 times (HR = 2.40; 95% CI = 1.36 – 4.24; p = 0.003).
Conclusions: Carriers of GPX1*ProPro genotype that have developed PC live shorter than those with at least one GPX1*Leu allele. Likewise, they have an increased risk of fatal outcomes, both independently and when combined with other prognostic risk factors. These results suggest that GPX1 rs1050450 gene polymorphism might be used as a prognostic biomarker in patients with prostate cancer.

P39

Investigation of hsa_circ_0044969 mediated regulation of PPM1D gene expression in breast cancer

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Keywords: PPM1D, circRNA, breast cancer, post-transcriptional gene regulation

Background: Breast cancer is one of the most common types of cancer. It has been published that its pathology has been associated with the dysregulation of numerous genes, environmental factors and various epigenetic mechanisms. Among the epigenetic factors, noncoding RNAs have emerged as important players in breast cancer pathology. The importance of circular RNAs, covalently closed noncoding RNAs, has increasead in recent years, since it has been understood that one of their functions is to sponge miRNAs and prevent them from binding to target mRNAs. PPM1D (protein phosphatase, Mg2+/Mn2+ dependent 1D) is one of the negative regulators of the cell stress response and was associated with aggressive tumors.
Materials and Methods: In this study, we aimed to investigate the expression profile of hsa_circ_0044969 and explore its potential role as a regulator of PPM1D through circRNA-miRNA-mRNA axis in breast cancer. MCF7 and MDA-MB-231 breast cancer cell lines, along with the normal breast epithelial cell line MCF10A, were used as study samples and the expression levels of has_circ_00449696 and PPM1D were measured by qRT-PCR.
Results: Both PPM1D mRNA and hsa_circ_00449696 are upregulated in breast cancer cell lines compared to normal breast epithelial. The expression profile of hsa_mir_1299 revealed downregulation only in MCF7 cells compared to normal.
Conclusions: Our results show that the expression levels of both hsa_circ_0044969 and PPM1D increase in a parallel way in breast cancer. Further investigation is required to elucidate the molecular mechanisms underlying this correlation, particularly within the circRNA-miRNA-mRNA regulatory axis.

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HRD testing doubled the number of ovarian cancer patients eligible for PARP inhibitor therapy

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Keywords: BRCA1, BRCA2, Ovarian Cancer, NGS, HRD, Olaparib

Background: Ovarian cancer remains one of the most lethal gynaecological cancers, with very few symptoms and a five-year survival rate of about 40%. Treatment options are scarce, consisting of surgery and platinum-based chemotherapy in the first-line setting, with potential PARP inhibitor use for patients with mutations in BRCA1/2 genes. In patients with ovarian cancer, since 2025 in Serbia, the deficiency of the homologous recombination repair pathway (HRD) is a predictive biomarker for the use of PARP inhibitors in newly diagnosed patients.
Materials and methods: The starting material was FFPE tissue from ovarian cancer patients sent for HRD testing from all health centers in Serbia. The total number of HRD tested patients was 203, and testing was performed in the Laboratory for Molecular Genetics at IORS or in the private molecular diagnostic laboratory – Genotypos Science Labs in Greece. For evaluating HRD status, we used the SophiaDDM Dx HRD solution kit that covers 28 HRR genes and evaluates the genomic instability signature (GIS) by lpWGS. GIS>0 and/or mutations in BRCA1/2 were considered HRD-positive samples. In the Genotypos laboratory, the AmoyDx HRD Focus Panel was used, which covers BRCA1/2 genes, and the cut-off for GSS was 50. Testing was performed by NGS using the NextSeq500 system (Illumina). Pair-end reading was used, and a cut-off of 5% for the variant allele frequency was applied with minimum coverage of 400x. The

Sophia DDM program, ANDAS Data Analyzer, and manual database searches were used for data analysis interpretation of pathogenicity.
Results: A group of 162 samples (79.8%) was tested at IORS, and 41 (20.2%) were outsourced. In 40 patients (19.7%), a BRCA1/2 deleterious variant was found, and those patients were classified as eligible for treatment with PARPi. Additionally, 47 patients (23.15%) who were BRCA1/2 WT were HRD-positive, were also eligible for the same treatment. Median GSS for AmoyDx was 29.6, and for SophiaDDM, the median GIS in the positive group was 7.2. In the cohort tested at IORS, we analysed alterations in other genes from the HRR pathway. TP53 gene status was described as WT in 9.87%, VUS in 16.67%, and deleterious in 73.46% of samples. The total number of deleterious variants found in other HRR genes was 67, with the highest frequency in PALB2 (24%), PTEN (15%), CHEK2 (13%), etc.
Conclusions: By implementing HRD testing in the diagnostic procedure, the number of patients who could benefit from PARPi treatment was doubled.

Acknowledgments and funding: We acknowledge AstraZeneca for donating the BRCA and HRD tests used in this nationwide testing. The study was designed, initiated, and conducted independently by Institute for Oncology and Radiology of Serbia without sponsorship or involvement from AstraZeneca.

SESSION 8

LIQUID BIOPSY

P41

Basic properties of extracellular vesicles from cerebrospinal fluid of subjects with non-malignant and malignant neurological disorders

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Keywords: brain cancer, extracellular vesicles, nanoparticle tracking analysis, sialic acid

Background: Cerebrospinal fluid (CSF) is a clear fluid with low protein content, widely used for both diagnostic and prognostic purposes. Although extracellular vesicles (EVs), small particles with a bilayer membrane, are present in CSF, data regarding their properties remain scarce. In this study, EVs were isolated from CSF of subjects with non-malignant (hydrocephalus) and malignant (medulloblastoma/ependymoma) neurological disorders to compare their concentration, size distribution, and surface presence of sialic acid-containing glycans. This study aims to provide preliminary data on the potential of basic CSF EVs properties as discriminating markers.
Material and Methods: EVs were isolated from pooled CSF samples of subjects with non-malignant (nmEVs) and malignant (mEVs) neurological disorders by differential centrifugation. Fluorescently labeled sialic acid-binding plant lectins: wheat germ agglutinin (WGA) and Sambucus nigra agglutinin (SNA) were used in nanoparticle tracking analysis (NTA) to assess EVs surface sialylation. The presence of tetraspanins, commonly used as EVs markers, was evaluated using antibodies against CD9, CD81, and CD63.
Results: Higher concentration of mEVs (1.08×10^{11} particles/mL) compared to nmEVs (1.50×10^{10} particles/mL) was revealed. Size distribution showed that the majority of EVs in both subject groups fell within the 100-200 nm range. The share of CD9-positive EVs was five times higher in nmEVs compared to mEVs, while the share of CD81-positive EVs did not differ notably between the two groups. Anti-CD63 antibody exhibited minimal reactivity to both nmEVs and mEVs. The share of WGA-reactive EVs was similar between nmEVs and mEVs, whereas the share of SNA-reactive EVs was over three times higher in mEVs.
Conclusions: An increased share of SNA-reactive mEVs, indicating changes in sialylation, was found. In addition, a reduced share of CD9-positive mEVs was observed. Alterations in basic CSF EVs properties may serve as potential markers of their origin.

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Circulating extracellular vesicles in liver and pancreatic cancer: an initial assessment of sialylation

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Keywords: Extracellular vesicles, hepatocellular carcinoma, nanoparticle tracking analysis, pancreatic cancer, sialic acid

Background: Extracellular vesicles (EVs) are cell-derived, membrane-surrounded vesicles that carry various bioactive molecules and deliver them to recipient cells. They are considered mini-maps of their cells of origin. Altered tissue/cell glycosylation is a hallmark of different cancers but little is known about how these changes affect circulating EVs. One of the most prevalent changes is sialylation, the addition of sialic acid (Sia) to galactose or N-acetylgalactosamine at the terminal position of glycan chains. Starting from data on the changes in sialylation of liver and pancreatic cancer tissues, this study aimed to assess whether these changes are mirrored in related circulating EVs, specifically within large EV populations, previously indicated to comprise cancer-derived vesicles. This is important issue regarding designation of introductory studies of the functional and biomarker potential of these EVs.
Materials and Methods: Serum samples were collected from patients with hepatocellular carcinoma (HCC, n = 5), pancreatic cancer (PC, n = 10), and healthy donors (HD, n = 10). Circulating EVs were isolated by differential centrifugation and ultracentrifugation. Their size distribution was analyzed using nanoparticle tracking analysis (NTA). Surface sialylation was assessed by fluorescent NTA (f-NTA) using Sambucus nigra agglutinin (SNA) and wheat germ agglutinin (WGA), which have distinct requirements for binding Sia residues. Statistical analyses were performed through GraphPad Prism 8.0 applying the Kruskal-Wallis test.
Results: Median (IQR) EV sizes from HCC (146 nm (144.5-150.8 nm)) and PC (155.5 nm (151.5-160.4 nm)) were significantly higher than those from HD (126.4 nm (123.3-128.9 nm)) ($P < 0.001$). Large EVs (>200 nm) were more abundant in cancers (~30%) compared to HD (~10%). There was no statistically significant difference ($P > 0.05$) in the sizes of SNA- and WGA-reactive EVs between cancers and HD. The proportion of SNA-reactive large EVs was similar between cancer patients and HD (~40%), whereas the proportion of WGA-reactive large EVs was similar between HCC and HD (~30%), but reduced in PC (~20%).
Conclusions: The median EVs size and the ratio of large EVs were increased in both HCC and PC in comparison to HD. This increase is accompanied by the changes in surface sialylation seen as decrease in sialic acid-binding lectins reactivity, notably in PC. The changes in EVs sialylation were opposite to those detected in liver and pancreatic cancer tissue.

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The association of baseline concentrations of circulating tumor DNA with clinical course of advanced melanoma

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Keywords: circulating tumor DNA, melanoma, prognosis

Background: Immune checkpoint inhibitors (ICI), applied as a monotherapy or in combination regimens, demonstrated significant survival benefits in patients with advanced melanoma, However, a significant proportion of patients fail to respond to ICI or experience relapse after an initially successful response. Detection and quantification of circulating tumor DNA (ctDNA) has been considered a promising prognostic biomarker in melanoma patients treated with ICI.

Since detection/quantification of low levels of disease in plasma is still challenging regarding analytical and clinical validations, we assessed association of baseline ctDNA presence/level with the clinical course of melanoma.
Patients and Methods: The study included 61 patients with BRAF-positive melanoma in clinical stage III/IV. All patients received ICI as adjuvant/neoadjuvant therapy. Cell-free DNA (cfDNA) was extracted from plasma samples using the MagCore Plasma DNA Extraction Kit (1.2 mL). CtDNA was quantified via BRAF V600 mutation using the QIAcuity Digital PCR System.
Results: Patients with detectable ctDNA more frequently experienced disease progression (p=0.06). The average fraction of variant allele (VAF) was significantly higher in patients who experienced disease progression (0.15 vs. 0.06; p=0.02). When VAF of 0.15 was used as cut off (also obtained by ROC analysis), superior duration of progression free survival and overall survival were observed in patients with VAF < 0.15 (median PFS 13.91 vs. 1.64 months, p=0.02; median OS 18.91 vs. 7.63 months, p=0.041).
Conclusions: The ctCNA presence/fraction at baseline may be considered as a marker of poor prognosis. In order to confirm these findings, a larger cohort of patients is needed to allow broader statistical analyses.

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P44

The use of pleural effusion from patients with lung cancer as a source of extracellular vesicles – first report from the EXPAND-EV project

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Keywords: Carcinoma, Non-Small-Cell Lung, Extracellular Vesicles, Pleural Effusion

Background: Pleural effusion (PE) is a frequent complication of lung cancer (LC), occurring in up to one-quarter of patients. Despite being routinely sampled for diagnostic cytology, its sensitivity is often insufficient, leaving a need for complementary molecular approaches. Small extracellular vesicles (sEVs) from PE are enriched in tumor-related proteins and RNAs and could serve as a form of liquid biopsy. Most studies have relied on ultracentrifugation (UC), but this method yields low-purity isolates, often unsuitable for proteomic profiling. Improved results were obtained by combining UC with size-exclusion chromatography (UC-SEC), while ExoProK introduced Proteinase K pretreatment to reduce contaminants. Nevertheless, clinically feasible and standardized protocols for PE-derived EVs are still missing. Within the EXPAND-EV EU project, we focused on EV isolation from PE of patients with advanced non-small cell lung cancer (NSCLC), evaluating methods which emphasize minimal input volume, short turnaround time, and compatibility with downstream omics.
Materials and Methods: sEVs from PE samples were isolated by three approaches: (1) an in-house spherical porous methacrylate-based polymer functionalized with VHH antibodies (chromatography-based method, CH), (2) UC, and (3) a commercial Norgen kit (CK). Vesicles were characterized using nanoparticle tracking analysis (NTA), transmission electron microscopy (TEM), atomic force microscopy (AFM), and flow cytometry for CD9 expression. Methods were compared for efficiency, purity, and clinical applicability.
Results: Both CH and CK enabled isolation from as little as 1 mL, while UC required ≥12 mL. CK was the fastest, followed by CH, whereas UC was time-consuming. CH yielded the highest particle counts and cleanest profiles, with UC producing the lowest yield and greatest protein contamination. TEM, AFM, and flow cytometry confirmed vesicular morphology and integrity, with CH isolates showing the highest purity.
Conclusions: CH and CK approaches are practical alternatives to UC, enabling efficient EV isolation from small PE volumes. CH isolates are suitable for proteomics, while CK combines speed with RNA recovery, supporting transcriptomic

analysis. LC-MS/MS profiling of PE-derived vesicles using the Norgen kit with additional pretreatment with Proteinase K (as in ExoProK) or a CK-SEC combination to enhance purity may help establish PE-derived EVs as a valuable source for biomarker discovery in LC.

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P45

Liquid Biopsy as a Tool in Guiding Anti-EGFR Monoclonal Antibody Rechallenge Therapy in Metastatic Colorectal Cancer

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Keywords: metastatic colorectal cancer (mCRC), liquid biopsy (LB), anti-EGFR antibodies, rechallenge therapy

Background: In patients with metastatic colorectal cancer (mCRC) who progressed to anti-EGFR monoclonal antibody (mAb) therapy, such as cetuximab and panitumumab, a rechallenge with the same agents is becoming a promising approach. Although patients demonstrate objective progression during the anti-EGFR treatment, emerging findings suggest that anti-EGFR-resistant clones decay, enabling the possibility of a rechallenge strategy (1). A key step for a deeper understanding of clinical progression in patients is the identification of mechanisms of secondary resistance to anti-EGFR mAb therapies. Limited data are currently available on the resistance mechanism that develops during the anti-EGFR treatment. Intratumoral heterogeneity is assumed to play a significant role in mCRC progression. During anti-EGFR therapy, subclones continuously arise through genomic mutations, resulting in faster disease progression and poorer overall survival (OS) in patients (2). Santini et al. (3) demonstrated that the reuse of cetuximab was a potentially effective strategy in patients with mCRC who had previously achieved clinical benefit from cetuximab-based therapy. A sufficiently lengthy treatment-free period, of a minimum of four and optimally longer than six months, should be provided. This study was the first to lay the groundwork for the hypothesis that acquired resistance to anti-EGFR therapy may be reversible and that, after a break from the therapy, the tumor may become sensitive again, which improves subsequent response and increases patient free survival (PFS). European and American health authorities have restricted the use of cetuximab and panitumumab, as mono- or in combination with chemotherapy, only to patients with wild-type (wt) RAS gene status (4). As with first-line treatment, confirmation of wt KRAS/NRAS gene status is recommended for anti-EGFR mAb reintroduction (5). Mutations in the BRAF gene are also recognized as negative prognostic markers in the response to the anti-EGFR therapy, and according to the European Society For Medical Oncology (ESMO) guidelines, testing for mutations in the BRAF gene is also recommended (6). The preferred method for selecting patients for the rechallenge strategy, but also for detecting mutations and monitoring secondary resistance, is liquid biopsy (LB). LB is a minimally invasive technique that shows promise in identifying various molecular changes crucial for achieving an effective response to treatment.
In our research, we aimed to analyze the distribution of mutations and characteristics of circulating tumor DNA (ctDNA) in plasma samples from patients who developed resistance to the anti-EGFR antibodies. This study may enhance our understanding of the mechanisms underlying the evolution of resistance to targeted therapies.
Materials and methods: Samples were collected from 25 patients (17 male/8 female), potential candidates for rechallenge therapy, over a period of 7 months. The median age of the study population was 64 years (range: 40-80 years). Among the 25 patients enrolled, 15 received cetuximab and 10 received panitumumab, before disease progression. Patients were included if the previous anti-EGFR therapy had been applied for at least 6 months. According to Santini's hypothesis (3), blood was sampled at least 4 months after receiving the last mAb-based therapy. The blood

was collected in anticoagulant tubes (EDTA/PAXgene tubes), from which plasma was separated by centrifugation at 2000g at 4°C for 10 minutes, according to the protocol. Circulating free DNA (cfDNA) was extracted using the QIAamp MinElute ccfDNA Mini Kit (Qiagen, Hilden, Germany) from a plasma volume of 4ml, and the concentration of cfDNA was measured using the Qubit™ 4 Fluorometer with the Qubit™ dsDNA HS Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). The extracted samples were subjected to mutation testing using the KRAS Mutation Test v2 (LSR) and/or BRAF/NRAS Mutation Test (LSR) (Roche Diagnostics, Mannheim, Germany), a qPCR-based method designed to detect somatic mutations in the KRAS, NRAS, and BRAF genes. Both tests are designed to include detection from DNA extracted from Formalin-Fixed Paraffin-Embedded (FFPE) tissue and blood. The KRAS Mutation Test v2 was designed to detect the following mutations: G12X and G13X in exon 2; A59X and Q61X in exon 3; A117X and A146X in exon 3. The BRAF/NRAS Mutation Test was intended to detect NRAS mutations in the same codons as well as in the KRAS gene, and also BRAF mutations in codons 469 (exon 11) and 600 (exon 15).

Results: The concentration of cfDNA was from 0.106 to 29 ng/µl. Variant allele frequency (VAF) values ranged from 10.5 to 23.5. In patients who had received cetuximab, 12 (80%) had at least one mutation, whereas 3 (20%) had no mutations in any examined genes. In the group of patients who had received panitumumab, 6 (60%) showed one or two mutations, whereas 4 (40%) did not harbor mutations in any of the examined genes. When considering both drugs, a total of 27 mutations in the KRAS and NRAS genes mutations were detected, in the following order for KRAS gene: 8 (29.63%) in codon 12, 7 (25.93%) in codon 61, 2 (7.41%) in codon 13, 1 (3.7%) in the codon 59, and also 1 (3.7%) in the codon 146. Also, for the NRAS gene, detected mutations were as follows: 6 (22.22%) in codon 13, 1 (3.7%) in codon 12, and 1 (3.7%) in codon 61. All wt KRAS patients were subsequently tested for the presence of NRAS and BRAF mutations. Of the 15 patients who received cetuximab, 8 (53.33%) showed at least one mutation in exon 2,3, or 4 in the KRAS gene. For four patients with detected KRAS mutations, a further analysis was performed for BRAF/NRAS genes to examine tumor heterogeneity. Only in one patient, who received cetuximab, four concurrent KRAS mutations (G12X, A59X, A146X, Q61X) were detected, three mutations in NRAS (G12X, G13X, Q61X), as well as a mutation in exon 11 of the BRAF gene. This was the only patient in the entire cohort with a confirmed BRAF mutation, accounting for 4% mutated BRAF of the total of 25 patients. Overall, when considering both drugs, as well as the presence of mutations in both genes, 18 (72%) patients had at least one mutation, while in 7 (28%) patients, no mutation was detected in the KRAS/NRAS/BRAF genes.

Conclusion: The initial results of our research suggest that NRAS gene mutations — particularly at codon 13 — are increasingly recognized as contributors to resistance in the context of rechallenge therapy. When examining tissue samples taken before the previous line of anti-EGFR therapy, the prevalence of NRAS mutations is notably low, ranging from 3% to 5% (7). Although these findings need validation in larger patient cohorts, they indicate that NRAS mutations could be a key factor in the development of resistance to the anti-EGFR treatments. The occurrence of multiple mutations in individual patients underscores the genetic heterogeneity of CRC, which represents a key hallmark of the disease and may play a pivotal role in the development of therapeutic resistance. The underlying mechanisms may involve alterations in the mutational landscape that promote the emergence of secondary resistance mutations, as well as selective pressure induced by anti-EGFR therapies, which allow previously minor clones to persist (8). Additionally, we observed that 72% of patients still harbor at least one mutation in the KRAS or NRAS gene even after 4 months without therapy. The higher percentage of mutations acquired after receiving cetuximab compared to panitumumab also provides an opportunity to further investigate the role of KRAS/NRAS/BRAF mutations in the acquisition of resistance to both drugs in patients with mCRC. Ultimately, rechallenging with the same anti-EGFR antibody-mAb-based therapy at the right time may offer new clinical benefits for mCRC patients, potentially delaying disease progression and improving treatment outcomes.

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SESSION 9

CLINICAL ONCOLOGY - BRIDGING PATIENTS AND RESEARCHERS

P46

Efficacy of Neoadjuvant Immune Checkpoint Inhibitors in Colorectal Cancer: A Retrospective Study

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Keywords: colorectal cancer, neoadjuvant immunotherapy, immune checkpoint inhibitors, MSI-H, pathological complete response

Introduction: This retrospective study evaluated the efficacy of neoadjuvant immune checkpoint inhibitors (ICIs) in patients with localized and locally advanced colorectal cancer (CRC). The research focused on microsatellite instability-high (MSI-H) tumors, which are known to respond well to immunotherapy. The study aimed to assess clinical and pathological responses, toxicity, and survival outcomes in this patient population.
Materials and Methods: From October 2020 onwards, 220 patients with localized or locally advanced CRC were screened, of whom 36 (16.4%) had MSI-H tumors. Among these, 24 patients (66.7%) received neoadjuvant immunotherapy, while 11 underwent surgery without neoadjuvant treatment, and 1 was excluded due to severe comorbidities. The cohort included patients with varying tumor stages (T2–T4, N0–N2) and locations (right/left colon, rectum). Immunotherapy regimens included nivolumab (54.2%), pembrolizumab (29.2%), prolgolimab (4.2%), ipilimumab+nivolumab (8.3%), and FOLFOX+nivolumab (4.2%). The median number of cycles administered was 5 (range: 2–31).
Results: Among all 24 treated patients, the objective response rates were: complete response (CR) in 12.5%, partial response (PR) in 33.3%, and stable disease (SD) in 45.8%. One patient (4.2%) experienced progressive disease (PD). Of the 16 patients who underwent surgery, 50% achieved a pathological complete response (pCR). Toxicity was manageable, with grade 1–2 adverse events occurring in 25% of patients, while one patient died due to severe toxicity (grade 5). At a median follow-up of 14.4 months, median progression-free and overall survival were not reached, with only one case of disease progression post-surgery.
Conclusions: Neoadjuvant immunotherapy demonstrated high efficacy in MSI-H CRC, with a notable pCR rate of 50% among operated patients. However, challenges remain, including the need for faster MSI testing and unresolved questions regarding the necessity of surgery for patients with clinical CR and the role of adjuvant therapy. These findings support further investigation into optimizing treatment protocols for this patient subset.

P47

Contribution of non-profit organizations and charity foundations to the development of comprehensive functional and psychological rehabilitation of cancer patients in cooperation with leading oncological institutions

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Keywords: cancer rehabilitation, cancer rehab, psychosocial rehabilitation, physical rehabilitation, non-profit organizations

Background: Most studies prove the special need for comprehensive physical, psychological and social rehabilitation for cancer patients. At the same time medical institutions accompany patient at the first stages: medical and early postoperative. In this regard, the involvement of non-profit organizations and charity funds in developing the accessibility of the oncorehabilitation system is extremely relevant.
Materials and methods: From 2019 to 2025 the study with a retrospective analysis includes projects and initiatives implemented by regional public non profit organization «Together we are stronger» for cancer patients on the basis of key medical oncological institutions of St. Petersburg.
Results: During the five-year period more than 15,000 cancer patients were covered by specialized programs in psychological, physical and social rehabilitation. Among the main implemented projects: weekly group classes in physical therapy and Scandinavian walking, weekly aquaerobic classes with a visit to gala cameras, regular individual and group classes with psychologists using various art therapy techniques (metaphorical cards, fairy-tale therapy, psychodrama, sand therapy etc.), as well as neurographs, psychological support of patients at their first appointment to cancer center, regular meetings with chefs and culinary master classes for cancer patients on proper nutrition, nutritional support and formation of food habits, as well as online vibrations with dietary and nutritionists doctors.
Additionally, 10 «field» programs of complex rehabilitation in groups combining both exercise therapy (Scandinavian walking, gypsum, yoga) and psychological training were conducted at sanatoriums of St. Petersburg.
Regular conferences in «Patients' school» format on treatment and recovery after cancer on the basis of key St.Petersburg medical institutions. In addition, «Together we are stronger» develops and publishes recommendations for cancer patients receiving drug treatment, radiation therapy, recommendations for nutritional support and balanced nutrition during anticancer therapy, as well as psychological manuals.
Conclusions: AThe involvement of non-profit organizations and charity funds in the process of comprehensive rehabilitation of cancer patients allows to create a continuous process of psychological, physical and social restoration and better informing for patients and their family regarding the diagnostics, treatment and rehabilitation after anticancer therapy.

P48

Dietary modulation of inflammation in breast cancer patients on aromatase inhibitors: randomized controlled trial

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Keywords: breast cancer, anti-inflammatory diet, cytokines, aromatase inhibitors, nutritional immunology

Background: Chronic inflammation is known to promote breast cancer progression and may modulate the effectiveness of endocrine therapies, including aromatase inhibitors (AIs). Cytokines, central to immune regulation and tumor microenvironment signaling, represent potential targets for intervention. Nutritional strategies aimed at reducing systemic inflammation may beneficially influence cytokine profiles and patient outcomes during AI therapy.
Patients and Methods: We conducted a 4-month randomized controlled trial involving 30 postmenopausal women with stage I–IIIa breast cancer undergoing adjuvant AI therapy for at least 6 months. Participants were randomized into two matched groups. The intervention group (n=16, mean age: 58.2±6.7 years) adhered to a structured anti-inflammatory diet enriched in omega-3 fatty acids, fiber, and antioxidants, and low in refined carbohydrates and saturated fats. The control group (n=14, mean age: 58.4±7.5 years) maintained their usual diet. Plasma cytokine levels were assessed at baseline and after 4 months via flow cytometry.
Results: After 4 months, the intervention group demonstrated significant reductions in circulating cytokines relative to controls: IL-18 (↓32%, p<0.001), IL-33 (↓9%, p<0.01), IFN-γ (↓28%, p<;0.05), and IFN-α2 (↓33%, p<0.01). Additional significant decreases were observed in TNF-α (↓39%), IL-6 (↓41%), IL-10 (↓41%), and IL-17A (↓49%) (p<0.05 for all). Furthermore, the intervention group experienced reductions in body mass index (BMI, −8.83%) and visceral fat area (−20.5%). No significant changes were detected in the control group.
Conclusions: An anti-inflammatory diet significantly reduced proinflammatory and interferon cytokines in breast cancer patients on AI therapy, accompanied by decreases in BMI and visceral adiposity. These results suggest that dietary modulation can mitigate systemic inflammation and potentially improve immune homeostasis during endocrine treatment. Further studies involving a larger number of patients are warranted to explore long-term clinical benefits and underlying mechanisms.

Acknowledgments and funding: This study was supported by the Science Fund of the Republic of Serbia (Grant PRISMA No.5050, Project title: Anti-Inflammatory Dietary intervention in breast cancer patients receiving aromatase inhibitors-AID).

P49

Predictive value of inflammatory ratios on the outcome of advanced melanoma patients treated with anti-PD1 monotherapy: A multicentric analysis

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Keywords: melanoma, immune-checkpoint inhibitors, inflammation, outcome

Background: Despite immune-checkpoint inhibitors (ICIs) transforming outcomes of melanoma patients, a vast number remain unresponsive to treatment. Baseline-derived host-related inflammatory ratios such as neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR) and monocyte-lymphocyte ratio (MLR) are easily accessible biomarkers that can depict the balance between myeloid-derived protumour inflammation and lymphocyte-derived antitumour response. Our analysis aims to assess the predictive value of baseline inflammatory ratios on the outcome of melanoma patients treated with mono-ICIs.
Patients and Methods: We conducted a multicentric retrospective analysis that included 112 advanced melanoma patients treated with mono-ICI (Pembrolizumab or Nivolumab) at three Centers in Serbia and Bosnia & Herzegovina. Data were extracted from the European Melanoma Registry (EuMelaReg). ROC curves with Youdens J-statistic were used to determine optimal cut-off-values (NLR: 3.35, PLR: 165.5, MLR: 165.5). The p-values regarding Progression-free survival (PFS and overall survival (OS) were calculated using log-rank (p<0.05; CI 95%).
Results: Each of the inflammatory ratios separately showed poorer prognosis in high-value subgroup in terms of shorter PFS (NLR: 3.55 vs 14.01- p<0.0001; PLR: 3.85 vs 11.48- p=0.0011; MLR: 5.16 vs 11.48- p=0.0171) and OS (NLR: 5.69 vs 23.59- p<0.0001; PLR: 6.12 vs 23.59- p<0.0001; MLR: 8.42 vs 21.78- p=0.0007). In group analysis, patients with all three ratios elevated were compared with patients with at least one low ratio. Shorter PFS (2.86 vs 10.56; p<0.0001) and OS

(6.78 vs 21.45; p<0.0001) were also observed in the all-high subgroup. Patients with high-value ratios were younger (p=0.0162). No difference was observed in terms of gender (p=0.99), BRAF mutation status (p=0.53), the occurrence of immune-related adverse events (p=0.34) or LDH levels (p=0.16).
Conclusions: Inflammatory ratios are promising and accessible biomarkers that, either alone or combined, could be incorporated into clinical practice delineating patients who will benefit the most from mono-ICI and those who would need additional agents or alternative therapeutic approaches.

P50

Validation of an LC-MS/MS method for the determination of imatinib and n-desmethyl imatinib in human plasma

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Keywords: drug monitoring, imatinib, LC-MS/MS, metabolite, plasma, validation

Background: Imatinib is a selective tyrosine kinase inhibitor used for the treatment of chronic myeloid leukemia and other malignancies. Its primary metabolite, N-desmethyl-imatinib, has similar pharmacological properties. Quantification of both compounds in plasma is essential for therapeutic drug monitoring and pharmacokinetic studies. In this study, an LC-MS/MS method for the determination of imatinib and N-desmethyl-imatinib in human plasma was developed and validated.
Materials and Methods: Chromatographic separation was performed on an InfinityLab Poroshell 120 EC-C18 chromatography column (4.6 × 100 mm, 2.7 µm) using a mobile phase consisting of methanol and water with 0.1 % formic acid and 0.2 % ammonium acetate (55:45, v/v). The column temperature was set to 40 °C and the flow rate of the mobile phase was 700 µL/min. The injection volume was 10 µL. Detection was performed in MRM mode, monitoring the transitions m/z 494 > 394 for imatinib and m/z 480 > 394 for N-desmethyl-imatinib. The total run time of the analysis was 5 minutes.
Results: For imatinib, the method showed excellent linearity in the range of 5–500 ng/ml (r = 0.999), with an intra-run accuracy of 104.59% and an inter-run accuracy of 105%. The precision ranged from 1.4 % to 5.67 % RSD, depending on the concentration. The LOD and LOQ were 1.2 ng/mL and 5 ng/mL, respectively. The recovery was 91%. For N-desmethyl-imatinib, linearity was also satisfactory (r = 0.998), with a within-run accuracy of 103.13% and a between-run accuracy of 105%. The precision ranged from 1.2% to 13.8% RSD. The LOD was 1.8 ng/ml and the LOQ was 5 ng/ml. The recovery was 89%.
Conclusions: The validated LC-MS/MS method is selective, accurate and precise and is suitable for routine analysis of imatinib and its metabolite in human plasma in accordance with ICH guidelines.

P51

Prevalence of head and neck cancer in Serbia

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Keywords: head and neck neoplasms, mouth neoplasms, incidence, mortality, epidemiology, Serbia

Background: Head and neck cancers represent a widely prevalent group of malignancies worldwide. Globally, they rank 16th in terms of incidence and 15th in terms of mortality. The aim of this study was to determine the descriptive epidemiological characteristics of head and neck cancers (ICD-10: C00–C13, C32) in Serbia during the period from 1999 to 2022.

Materials and Methods: Data were obtained from the Cancer Registry of Central Serbia (1999–2015) and the Republic of Serbia Cancer Registry (2016–2022). All registered cases of head and neck cancers were included, based on the classification of the International Agency for Research on Cancer (ICD- 10: C00–C13, C32). A descriptive epidemiological method was applied. Incidence and mortality rates were presented as age-standardized rates (per 100,000 population), using direct standardization based on the World Standard Population.
Results: In 1999, women were 5.3 times less likely to develop and 8.5 times less likely to die from head and neck cancers compared to men. However, this disparity has significantly decreased over time — by 2022, women were 3.7 times less likely to develop and 3.6 times less likely to die from these cancers. The difference is even more pronounced in the case of oral cancer. Two and a half decades ago, women were six times less likely to die from oral cancer, whereas in 2022, female mortality from oral cancer was only half that of men. Among men, age-standardized incidence rates remained relatively stable over time (20.3 per 100,000 in 1999 vs. 20.0 in 2022), whereas in women, a notable increase was observed (from 3.8 in 1999 to 5.3 in 2022). Age-standardized mortality rates in men showed a slight downward trend (from 11.9 in 1999 to 9.1 in 2022), while in women, an upward trend was evident — increasing from 1.4 in 1999 to 2.5 in 2022. The highest number of cases occurred in individuals over the age of 50, with a noticeable trend toward increasing incidence among younger age groups over time.
Conclusions: Between 1999 and 2022, Serbia experienced a marked increase in both the incidence and mortality of head and neck cancers among women. These results highlight the critical importance of reinforcing preventive healthcare measures and advancing early detection programs, particularly targeting high-risk populations.

P52

Different financing mechanisms of innovative therapies in oncology: a scoping review

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Keywords: financing, innovative therapies, oncology, scoping review , managed entry, value-based agreements

Background: Innovative oncology therapies, such as gene and cell-based treatments, targeted agents, and advanced immunotherapies, present unique financial challenges for healthcare systems globally, due to high upfront costs, uncertain long-term outcomes, and evolving regulatory and reimbursement landscapes. The aim of this research was to systematically characterize the landscape of EU financing strategies for oncology innovation in therapies. This approach is needed to systematically map the full spectrum of financing strategies, clarify key concepts and definitions, identify emerging models, and highlight gaps in evidence and implementation. Unlike conventional reviews, a scoping review offers a broad, structured overview without restricting to study design or quality, which is ideal for exploring this complex and rapidly evolving field.
Methods: As a comprehensive search strategy, the scoping review was implemented across multiple bibliographic databases and grey literature sources to identify studies on financing innovative oncology therapies. Data were iteratively charted, capturing study characteristics, financing models, outcomes, and contextual factors. Findings were synthesized descriptively, mapping key themes, regulatory and funding mechanisms, evidence gaps, and emerging models, without formally appraising study quality.
Results: Scoping review identified that the oncology financing ecosystem includes a mosaic of value-based contracts, specialized funding mechanisms, and patient financial supports. The existing literature on financing models is fragmented, encompassing varied mechanisms such as outcome-based agreements, managed entry deals, public–private funding. across different national and institutional contexts. However, the field lacks consolidated evidence for comparative effectiveness, long-term affordability, and transferability across different health systems, which are all specific.
Conclusions: There are several successful prototypes for financing innovative oncology treatments that can help guide future policy development and research agendas within countries and their healthcare systems. The challenge lies in effectively scaling these models, generating stronger evidence of cost-effectiveness and patient benefit, and ensuring equitable access. Integrating financing strategies into national health plans, empowering provider-led models, and evolving investment mechanisms might be the key to transforming oncology care sustainably.

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P53

Germline *BRCA1* and *BRCA2* Testing in the Context of Personalized Therapy: National Experience from Serbia in Breast Cancer Management

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Keywords: *BRCA1, BRCA2*, NGS, Breast Cancer, Olaparib

Background: Breast cancer is the most common cancer in women, and germline *BRCA1/2* mutations significantly increase lifetime risk, guiding both risk assessment and treatment. PARP inhibitors, like olaparib, exploit defective DNA repair in BRCA-mutated cells, improving progression-free survival in HER2-negative patients. This study aimed to implement nationwide BRCA testing in Serbia, enabling personalized therapy for eligible patients. It also marked the first collaborative effort of the three major Serbian institutions to introduce a unified NGS protocol for BRCA testing.
Patients and Methods: From April 2024 to June 2025, a total of 1,739 patients were tested across three institutions: Institute for Oncology and Radiology of Serbia, Oncology Institute of Vojvodina, and Institute of Molecular Genetics and Genetic Engineering. *BRCA1/2* mutation analysis was performed using next-generation sequencing on the Illumina platform with the Devyser BRCA NGS kit. Genomic DNA extracted from peripheral blood was quality-controlled and subjected to massive parallel sequencing. Data analysis was conducted using Amplicon Suite software.
Results: Most patients (72.5%) underwent testing at an early stage of disease. HR+/HER2− breast cancer was the predominant subtype (63.7%). Pathogenic or likely pathogenic variants were detected in 97 patients (5.6%), including 56 *BRCA1* (3.2%) and 41 *BRCA2* (2.4%) mutations. Variants of uncertain significance were identified in 42 cases (2.4%). Among *BRCA1*-positive patients, 76.8% presented with triple-negative breast cancer (age range: 32–70 years; mean age: 50 years). In contrast, *BRCA2*-positive cases were predominantly HR+/HER2− (65.8%; age range: 34–78 years; mean age: 53.5 years). A notable variation in the geographical distribution of *BRCA1/2* mutations was observed, with prevalence rates of 3.2% in southern Serbia, 5.0% in central Serbia, and 8.35% in Vojvodina.
Conclusions: The introduction of olaparib for breast cancer treatment in Serbia strengthens precision oncology by offering an additional targeted therapy. Nationwide collaboration among major institutions has enabled the implementation of germline BRCA testing, which is crucial for identifying patients eligible for PARP inhibitors and for bringing personalized medicine into routine clinical care. Ongoing research should assess real-world outcomes in patients treated with olaparib, while this germline testing will also provide valuable insights into the genetic landscape of the Serbian population.

Acknowledgments and funding: We acknowledge AstraZeneca for donating the BRCA tests used in this nationwide testing. The study was designed, initiated, and conducted independently by Institute for Oncology and Radiology of Serbia, Oncology Institute of Vojvodina, and Institute of Molecular Genetics and Genetic Engineering without sponsorship or involvement from AstraZeneca.

SESSION 10

BIOINFORMATICS AND CANCER GENOMICS

P54

Analysis of COMT-211 and AMPD3-206 transcripts in lung cancer

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Keywords: biomarkers, lung cancer, lncRNAs

Background: Lung cancer (LC) is often diagnosed at advanced stages, and there is an urgent need to develop new tools for early disease detection and management strategies. Recent pan-cancer transcriptome analysis has revealed increased expression of COMT-211 and AMPD3-206 transcripts in malignant compared to non-malignant lung tissue. This study aimed to analyze the expression of COMT-211 and AMPD3-206 in LC using in silico and in vitro approaches to evaluate their potential as biomarkers for disease detection.
Material and Methods: The coding potential, subcellular localization, and secondary structure of the transcripts were predicted using available online tools. The UCSC Xena Browser platform was utilized to compare transcript expression levels between malignant and non-malignant lung tissue samples, incorporating data from the GTEx and TCGA datasets. Relative expression levels of COMT-211 and AMPD3-206 were quantified by qRT-PCR in the following lung cell lines: malignant A549 and CRL2066, and non-malignant BEAS-2B.
Results: COMT-211 and AMPD3-206 were predicted to be non-coding transcripts (with coding probabilities of 3.5% and 39.1%, respectively), predominantly localized in the cytoplasm, with COMT-211 showing a more stable secondary structure than AMPD3. The results from the Xena Browser platform indicated a significantly increased expression of both transcripts in primary tumor tissues compared to adjacent non-tumor tissues of patients with LC, as well as to normal lung tissues of healthy individuals. While the expression of AMPD3-206 was similar between adjacent non-tumor tissues of patients with LC and lung tissues of healthy individuals, a significant increase in the expression of COMT-211 was observed in adjacent non-tumor tissues of patients with LC. Although COMT-211 was detected in all analyzed lung cell lines, its expression was extremely low. In contrast, the expression of AMPD3-206 was higher, but no significant differences were observed between the cell lines ($p>0.05$).
Conclusions: COMT-211 and AMPD3-206 transcripts may represent long non-coding RNAs. The upregulation of COMT-211 in both tumor and adjacent non-tumor tissues, along with its structural stability, suggests its potential as an early marker of tumor-associated changes or the tumor microenvironment. In contrast, AMPD3-206 shows expression patterns consistent with a tumor-cell origin. Further studies are needed to clarify their origin and biomarker potential in lung cancer.

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P55

In Silico Characterization of the Non-Coding Transcript BUD23-212 in Gastrointestinal Cancers

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Keywords: Gastrointestinal Neoplasm; Gene Expression Regulation, Neoplastic; MicroRNAs; RNA, Long Noncoding; Transcriptome

Background: Recent pan-cancer transcriptomic analyses have identified differential activity of two alternative promoters of the BUD23 gene in malignant versus non-malignant gastrointestinal (GIT) mucosa. The promoter upregulated in tumor tissues drives the expression of the transcript isoform BUD23-212 (ENST00000453316), suggesting a potential role in malignant transformation. This study aimed to predict the functional relevance of BUD23-212 in gastrointestinal cancers using an in silico approach.
Methods: We employed publicly available in silico tools to evaluate the transcript's coding potential, subcellular localization, repetitive element content, and miRNA binding interactions. Transcript expression profiles in tumor and non-tumor samples from the esophagus, stomach, colon, and rectum were retrieved from the UCSC Xena browser. Additionally, we assessed BUD23-212 expression in non-malignant HCEC1CT cells and malignant cell lines HCT116, DLD1, SW620, and DLD1R using our transcriptomic dataset.
Results: CPC2 classified BUD23-212 as non-coding with high probability. According to AnnoLnc2, the transcript contains Alu/SINE repetitive elements, which are often enriched in non-coding RNAs, and confer regulatory and structural functions. lncLocator predicted BUD23-212 enrichment in the nucleus and ribosomes, suggesting potential roles in epigenetic regulation, RNA processing, or micropeptide translation. miRBase and miRDB predicted binding sites for miR-1285-2, miR-509 isoforms, and miR-4308, microRNAs with established tumor-suppressive roles in gastric and colorectal cancers, suggesting that BUD23-212 may function as a competitive endogenous RNA (ceRNA) or molecular sponge. According to UCSC Xena data, BUD23-212 expression was significantly elevated in the tumor compared to non-tumor GIT tissues. Our transcriptomic data confirmed expression in malignant cell lines HCT116 and DLD1R.
Conclusions: BUD23-212, which is overexpressed in malignant GIT tissues and analyzed cancer cell lines, appears to exert its molecular function as a regulatory non-coding RNA. Future research should aim to clarify the precise molecular functions of BUD23-212, explore its potential as a biomarker, and investigate the therapeutic potential of its targeted silencing in gastrointestinal cancers.

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P56

Comparative analysis of machine learning algorithm results in predicting the probability of rectal tumor presence

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Keywords: algorithms, computed tomography, machine learning, rectal carcinoma

Background: This study examines the potential applications of machine learning (ML) algorithms in the analysis of computed tomography (CT) scans aimed at diagnosing tumor changes in specific organs. The focus of this study is on detecting tumor tissue in the rectal area. The aim is to train a model that will recognize and identify the tumor location using exclusively CT scans, without relying on magnetic resonance imaging. The long-term goal is the development and validation of algorithms that could be integrated into medical devices for automatic identification of tumor tissue,

where the device would display the probability that the tissue is affected by a tumor.
Material and Methods: The study included data from 140 patients, among whom were patients with healthy rectums. In 130 patients, a tumor localized entirely in the rectum was diagnosed, while 10 patients with healthy rectums were included to balance the dataset. Only those slices depicting the rectal region were selected, resulting in approximately 3,600 images suitable for analysis. A set of the most relevant features was extracted from the images, and a table was created for training and testing multiple ML algorithms. The algorithms used were logistic regression (LR), linear discriminant analysis (LDA), support vector machines (SVM), classification and regression trees (CART), naive Bayes (NB), and k-nearest neighbors (KNN). In addition to classical ML algorithms, neural networks were trained to compare performance and explore the potential of deep learning in tumor detection. Models were trained on the same feature set with a training and testing split. Evaluation focused particularly on sensitivity and specificity parameters, which are critical in medical diagnostics.
Results: The best result on the available dataset was achieved using the SVM algorithm, which reached over 80% accuracy in tumor area detection. Neural networks demonstrated potential for higher sensitivity, with a need for further model architecture tuning to improve specificity.
Conclusions: This approach can contribute to more efficient diagnostics, save resources and time for physicians, as well as enabling more precise therapy planning and a personalized patient approach. Furthermore, potential integration of the developed algorithms into medical equipment could improve automatic and rapid tumor detection during routine diagnostic procedures.

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P57

Bioinformatic evaluation of SMTN promoter-driven transcripts overexpressed in gastrointestinal cancers

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Keywords: RNA, micropeptides, gastrointestinal neoplasms

Background: Recent evidence suggests that some non-canonical transcripts harbour small open reading frames (sORFs) that encode microproteins with potential functional significance. Smoothelin, encoded by the SMTN gene, is a cytoskeletal protein composed of 915 amino acids, primarily expressed in differentiated smooth muscle cells. Transcripts SMTN-206 (ENST00000422839) and SMTN-209 (ENST00000432777) code for proteins 37 and 91 amino acids long, respectively. Recent pan-cancer transcriptome analysis has revealed that the activity of the promoter driving their expression is significantly increased in gastrointestinal tumours.
Material and Methods: Expression of SMTN-206 and SMTN-209 in tumor and non-tumor tissue was investigated using TCGA and GTEx datasets via the USCS Xena Browser. Sequences of SMTN-206 and SMTN-209 were retrieved from the Ensembl GRCh38 genome browser in FASTA format. Analyses included predictions of transcript localization, secondary structure, and interactions with miRNA. Additionally, sORF detection and microprotein localization were performed for SMTN-206. Ribosome profiling (RiboSeq) data were obtained from the GSE269371 dataset from NCBI Gene Expression Omnibus and used for estimating ribosome occupancy in CaCo2 and HCEC-1CT cell lines.
Results: Expression data revealed expression of SMTN-206 and SMTN-209 in colon, rectum and stomach tumors, suggesting tissue-specific promoter utilization. SMTN-206 demonstrated significant differential expression between tumor tissue and healthy gut mucosa warranting its prioritization for further analysis. MicroRNA interaction predictions indicated associations with miRNAs involved in tumor suppression and immune regulation. sORFs detection confirmed a translated region located between nucleotides 456-566 producing microprotein with extracellular localization. RiboSeq data confirmed differential ribosome occupancy between tumor-derived CaCo2 and non-tumor HCEC-1CT cell lines, suggesting increased translation in tumor cells.
Conclusions: Given its tumor-specific expression, increased translation in tumor cells, and interactions with cancer-relevant miRNAs, SMTN-206 emerges as a promising biomarker candidate in GI tumors. The encoded microprotein warrants further investigation due to its significant structural divergence from the canonical protein and its potential

role in tumorigenesis.

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P58

Comprehensive analysis of lysyl oxidase family gene expression and prognostic significance in colorectal cancer

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Keywords: colorectal neoplasms, hypoxia, biomarkers, prognosis, molecular targeted therapy

Background: Colorectal cancer (CRC) remains a major cause of cancer-related mortality despite advances in diagnosis and therapy. Given its clinical and molecular heterogeneity, novel biomarkers and therapeutic targets are urgently needed. The lysyl oxidase (LOX) family, comprising LOX and four LOX-like proteins (LOXL1–4), plays a central role in extracellular matrix remodeling via collagen and elastin cross-linking. Emerging evidence implicates LOX family members in tumor proliferation, invasion, and metastasis; however, their prognostic significance and mechanistic roles in CRC remain poorly defined. Therefore, we aimed to conduct a comprehensive analysis of the LOX family in colorectal cancer.
Materials and Methods: We conducted a comprehensive analysis of LOX family gene expression using multiple independent colorectal cancer (CRC) datasets from the GEO database. Associations with clinicopathological features, molecular subtypes, and survival outcomes were systematically evaluated. Gene set enrichment analysis (GSEA) was performed to explore potential biological pathways associated with the most relevant LOX genes. The key candidate genes were further validated in CRC cell lines compared to normal colonic epithelium using quantitative real-time PCR (qRT-PCR).
Results and Conclusions: Most LOX family members exhibited dysregulated expression in CRC, with LOX and LOXL2 showing the most consistent and robust upregulation across multiple datasets. Elevated expression levels of several members were significantly associated with advanced tumor stage, and poorer prognosis. Notably, LOX and LOXL2 expression was enriched in right-sided CIMP-high, MSI-H, and BRAF-mutant tumors. These two genes emerged as the most promising candidates and were selected for further analysis. GSEA revealed that high LOX and LOXL2 expression was strongly associated with the activation of epithelial–mesenchymal transition (EMT), hypoxia, IL2–STAT5 signaling, and inflammatory pathways, with a shared EMT-related gene signature observed across datasets. Subsequent validation confirmed significant upregulation of LOX and LOXL2 in CRC cell lines compared to normal colonic epithelium ($p < 0.05$). Together, these findings suggest that LOX and LOXL2 may drive aggressive, mesenchymal-like phenotypes in CRC and represent promising diagnostic biomarkers or therapeutic targets, particularly in inflammation-associated molecular subtypes.

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P59

The critical role of ADAMTS8 expression deregulation in forwarding vascular invasion in rectal carcinoma

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Keywords: rectal carcinoma, vascular invasion, ADAMTS8

Background: Rectal carcinoma (RC) accounts for approximately 30% of all colorectal carcinomas (CRC) and is recognized as a distinct clinical entity with unique causes and treatment strategies. Aside from lymphatic invasion, RC can spread in several vascular ways previously identified and validated as prognostic markers. Vascular invasion (VI) has consistently been shown to be associated with poor prognosis, both when detected by pathology and radiology methods, however, the molecular basis of VI is poorly studied. Several mechanisms may contribute to the VI process, including epithelial-mesenchymal transition (EMT), remodeling of the extracellular matrix (ECM), and angiogenesis. This integrative study was conducted with the aim of identifying genes and pathways specific for a VI-positive rectal adenocarcinoma phenotype.
Material and Methods: Using cBioPortal dataset platform, we examined mRNA expression and methylation data of 41 pre-selected, literature-based genes proven to be involved in regulating EMT, ECM, and angiogenesis during colorectal carcinogenesis and their potential association with vascular invasion as a clinical feature in a RC dataset consisting of 78 samples, which were filtered from the COADREAD TCGA (Firehose Legacy) dataset.
Results: Among 41 tested gene candidates, only ADAMTS8 showed a significant association between lower ADAMTS8 mRNA expression and vascular invasion was detected (p=0.039, tested by Mann-Whitney U test). In addition, methylome analysis strongly suggests promoter methylation of the ADAMTS8 gene, as a mechanism of epigenetic gene silencing, since in this cohort ADAMTS8 mRNA expression significantly correlates with ADAMTS8 methylation level (p=0.012, r=-0.263, Spearman's test of correlation). Moreover, we observed a tendency toward association between higher level of ADAMTS8 methylation and vascular invasion (p=0.087, tested by Mann-Whitney U test). Interestingly, protein-protein interaction analysis among protein products of tested 41 genes on STRING platform revealed that only ADAMTS8 protein formed a distinct cluster, displaying no interactions with any other queried proteins. Observed associations require further functional and experimental analysis to elucidate the exact role of ADAMTS8 expression deregulation in forwarding vascular invasion RC.
Conclusions: Obtained results have potential clinical utility, providing new approaches for developing targeted therapies for VI-positive rectal cancer, and require in vitro validation.

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P60

Deep learning on combined multistain and color-depth histology enhances early breast cancer prognosis

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Keywords: breast cancer, deep learning, distant metastasis, histopathology, microscopy, multicolor-depth, multistain, pan-cytokeratin, prognosis, ResNet

Background: Traditional clinicopathological markers of breast cancer outcome are now supplemented by molecular assays such as gene-expression panels, microRNA signatures, circulating tumour cells, proliferation markers, cytokines,

and stem-cell markers. These molecular tools often outperform classical markers but still leave considerable prognostic uncertainty. Because outcome prediction in early breast cancer has the potential to guide adjuvant therapy, we sought to build a deep-learning pipeline that delivers superior accuracy compared with existing approaches. Rationale and Aim Classification of histopathology images by use of convolutional neural networks has typically been approached by a single stain and a single image format. We reasoned that complementary stains and multiple colour-depth representations would expose richer morphology and therefore boost prognostic power. We focused on early-stage disease, where accurate risk stratification can spare low-risk patients from overtreatment and recommend high-risk patients toward intensified therapy. Our goal was to predict distant metastasis with maximal accuracy.
Materials and Methods: Whole-slide tumour sections from early breast cancer patients underwent two laboratory stains: 1. AE1/AE3 pan-cytokeratin (pan-CK), highlighting epithelial malignant patches. 2. Hematoxylin and eosin (H&E), visualising global histology. Slides were scanned and exported in three image formats: RGB colour, 8-bit grayscale and binary masks produced at five intensity thresholds, yielding seven biologically distinct datasets per patient. The final training set thus comprised 2,646 histopathological tumour images. Deep-Learning Pipeline A pretrained ResNet-50 served as a feature extractor and classifier. We implemented experimental data augmentation rather than the traditional virtual approach. The data augmentation was thus derived from physically different stains and image-depth transformations, not from synthetic flips or rotations. Strict separation of development and test sets prevented data leakage and ensured an unbiased estimate of performance.
Results: Single-dataset performance • Grayscale pan-CK: accuracy 94.4 % AUC 0.982 • Grayscale H&E: accuracy 85.7 % AUC 0.992 Pooled multistain–multidepth model Training on all 14 datasets (two stains x 7 image depths) pushed performance to 100 % accuracy and an AUC of 1.000 on the test set, confirming that heterogeneous visual inputs supply complementary prognostic information. Discussion Our findings show that microscopic morphology still holds untapped prognostic information that is only revealed when a deep learning network is provided with multiple stains and colour depths. Experimental augmentation with pan-CK and H&E stains provided biologically meaningful diversity. Grayscale images provided better discriminative performance than full-colour or binary images, yet the merged model still benefited from every representation. Previous cancer studies combining stains report similar gains, but to our knowledge, none have combined multiple staining with systematic colour-depth variation and binary thresholding. Our work supports expanding histopathology pipelines beyond H&E staining and RGB imaging to capture more comprehensive information about a tumour.
Conclusions: Conclusions and Outlook Deep transfer learning on complementary stains and colour-depth representations provides better prognostic accuracy for early breast cancer than single-stain models or traditional clinicopathological markers. This strategy can be integrated with radiomics, genomics, proteomics, and laboratory data to further refine personalised therapy. Because patients with early disease have the widest treatment choices, they stand to gain the most from the precise risk estimates offered by this multistain, multidepth deep-learning framework.

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Exploring the Structural Impacts of Missense Variants in USP7 from the perspective of effective inhibitor selection and MDM2-p53 coordination via classical MD simulations and docking studies

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Keywords: USP7, p53/MDM2, AutoDock Vina, Molecular Docking, Molecular Dynamic Simulation

Background: Ubiquitin-specific protease 7 (USP7) is a deubiquitinating enzyme involved in the regulation of multiple signalling pathways. One of the crucial roles of USP7 is the regulation of the tumour suppressor p53 together with MDM2. Under normal physiological conditions, USP7 indirectly suppresses p53 by stabilizing MDM2, but it stabilizes p53, thereby inducing apoptosis, under genotoxic stress. The presence of certain USP7 mutations causes excessive MDM2 stabilization, which triggers p53 degradation and therefore allows cancer cells to escape apoptosis. Due to this

paradoxical role of USP7 in p53 regulation together with its importance in ubiquitination processes, it is a critical target for the development of novel therapeutic strategies in cancer.

Materials and Methods: Within the scope of this study, we aimed to explore the possible structural impacts of missense variants in USP7 on the coordination of MDM2-p53 binding together with the selection of effective inhibitors. For this purpose, we utilized TCGA database to select the missense variants in USP7 with a pathogenity score >70%, already associated with the solid cancer. By using VMD tool, we prepared mutant and native USP7 systems that were further subjected to classical Molecular Dynamic simulations with CHARMM parameters. Molecular Dynamic simulations were collected as NpT ensemble for 100 ns. Then, the impacts of the pathogenic missense variants on USP7-ubiquitin aldehyde and USP7-inhibitor binding were evaluated via protein-protein and protein-small molecule docking studies.

Results: Depending on the results of 100 ns MD simulations, we have reported that 10 out of 24 variants were leading to significant change in the structural properties of USP7 by causing thermodynamic instability. Also, the presence of several variants was leading to competitive binding of specific USP7 inhibitors towards the ubiquitin aldehyde, known as a natural ligand, to keep USP7 in active conformation. Therefore, the required structural stability for its effective function was not achieved by USP7 due to the lack of, or partial interaction with ubiquitin aldehyde. Within the literature, 15 different small molecules serving as USP7 inhibitors were selected to evaluate their binding potential and effectiveness towards USP7-mutant complexes via AutoDock Vina docking tool. Among many, the binding performances of XL177A, XL188, XL203C, ALM4, ALM5, FLX4, L55, USP7-055 and USP7-797 inhibitors towards USP7 were superior even in the presence of many missense variants in USP7. Among these, the best performance was reported for ALM4, ALM5, FLX4 and L55 small molecules in terms of effective inhibition of USP7 even in the presence of many missense variants. We also reported the impacts of missense variants existing in USP7-TRAF domain on the dynamics of MDM2-p53. It was reported that R66C variant resulted in the loss of p53 coordination, by up to 30% while maintaining MDM2 coordination due to the loss of structural flexibility within TRAF domain.

Conclusion: The data obtained in this study highlight the importance of constituting theoretical background about the mutation-specific structural and functional changes for the effective inhibitor selection for USP7.

SESSION 11

NOVEL CANCER THERAPIES AND DRUG DEVELOPMENT STRATEGIES

P62

New bis-pyrazolate zinc(II) complexes as potential anticancer drugs: from structure to anticancer activity

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Keywords: DNA binding, cytotoxicity, apoptosis

Background: Zinc(II) plays a vital role in biological systems due to its unique properties: it is redox-inactive, a strong Lewis acid, and supports flexible coordination. Unlike redox-active metals, Zn(II) does not generate free radicals, contributing to antioxidant protection. It is involved in over 3000 human proteins, essential for DNA/protein synthesis, immune response, and brain function.1,2 Zinc imbalance is linked to cancer; chelation is used in excess, while ionophores help restore levels in deficiency. Recent heterometalllic Pt-L-Zn complexes show higher cytotoxicity than cisplatin, likely due to their interaction with multiple cellular targets.
Material and Methods: The binding affinity of the studied complexes with CT-DNA via intercalation was determined using fluorescence spectroscopy while with human serum albumin (HSA) was determined through fluorescence quenching experiments. The influence of different zinc(II) complexes on colon cancer HCT-116 and pancreatic cancer MIA PaCa-2 (obtained from the American Type Culture Collection, USA) cells viability was investigated, by measuring the intensity of purple formazan in control and treated cell samples (MTT assay). The type of cell death induced by different zinc(II) complexes was determined by the fluorescent dye acridine orange (AO, obtained from Acros Organics, New Jersey, USA), and ethidium bromide (EB, obtained from SERVA, Germany). The quality and quantity of RNA were evaluated using a BioSpec-nano spectrophotometer (Shimadzu Scientific Instruments). The generated cDNA was used as a template for gene expression analysis using quantitative real-time PCR (qRT-PCR).
Results: Three novel Zn(II) complexes [ZnCl2(H2LtBu)], [ZnCl2(Me2LtBu)] and [Zn2Cl4(H2LCatBiPyPh)2] (where H2LtBu is 2,6-bis(5-tert-butyl-1H-pyrazol-3-yl)pyridine, Me2LtBu is 2,6-bis(5-tert-butyl-1-methyl-1H-pyrazol-3- yl)pyridine and H2LCatBiPyrPh is 1,2-bis((5-phenyl-1H-pyrazol-3-yl)methoxy)benzene) were synthesized and characterized. Structural analysis revealed different coordination geometries: distorted trigonal bipyramidal ([ZnCl2(H2LtBu)]) and tetrahedral ([Zn2Cl4(H2LCatBiPyPh)2]). The complexes were examined for their potential antitumor activity. The complexes showed moderate binding to CT-DNA and human serum albumin (HSA), with DNA-binding affinity in the order: $[ZnCl_2(Me_2LtBu)] < [Zn_2Cl_4(H_2LCatBiPyPh)_2] < [ZnCl_2(H_2LtBu)]$. Cytotoxicity tests demonstrated significant antiproliferative effects against HCT-116 and MIA PaCa-2 cancer cells, with $[ZnCl_2(H_2LtBu)]$ being the most active than cisplatin in pancreatic cancer cells after 72 h. especially in inducing apoptosis. Gene expression studies revealed downregulation of TP53 (homo sapiens tumor protein p53) across all complexes, while $[ZnCl_2(H_2LtBu)]$ also reduced CASP3 (Caspase 3) and IGF1R (insulin-like growth factor 1) expression, indicating interference with apoptosis and cell proliferation.3-5 These findings suggest the complexes have potential as anticancer agents and warrant further molecular-level investigation.
Conclusions: Among investigated complexes $[ZnCl_2(H_2LtBu)]$ showed the most pronounced induction of apoptosis. Gene expression analysis suggested activation of apoptotic and necrotic signaling pathways, warranting further studies at transcriptomic and proteomic levels.

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First experience of using intraluminal photodynamic therapy with pulse-mode radiation of gastrointestinal tract and respiratory system tumors

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Keywords: photodynamic therapy, pulse-mode radiation, gastrointestinal tract and respiratory system tumors

Background: For patients with inoperable tumor gastro-intestinal tract and respiratory system tumors using intraluminal photodynamic therapy and calculated regime of pulse-mode radiation shows a higher antitumor effect compared to the standard persistent irradiation by reducing photo-induced hypoxia of the tumor tissue.
Materials and methods: From August, 2019 to May 2025, two groups (I and II group) of patients underwent the treatment. Group I consisted of three subgroups: 33 patients with gastrointestinal tract tumor. Nineteen patients in Group I had stenosing inoperable gastrointestinal tumors and complaints of dysphagia of grade 2–3 (subgroup I(a)); six patients had inoperable gastrointestinal tumors without signs of stenosis (subgroup I(b)); and eight patients had stage I gastrointestinal tumors (subgroup I(c)). Group II included 7 patients with bronchial tumors: two patients had stenosing inoperable lung cancer with grade 2–3 respiratory failure; one patient underwent non-radical resection of a lung tumor; three patients had grade I bronchial tumors; and one patient had a local recurrence of a laryngeal oropharyngeal tumor after a radical course of external beam radiation therapy.
Results: In Group I subgroup I (a) in 15 of 19 patients (79%) with a stenotic gastrointestinal tumor, 2-3 days after intraluminal photodynamic therapy during pulse-mode radiation, experienced relief from dysphagia, and independent feeding with solid food became possible. Control esophagogastroduodenoscopy showed partial tumor regression and 3-9 mm expansion. In the subgroup I (b) in 5 cases (83 %) endoscopically observed partial regression, in 1 patient (17 %) – complete regression. In Group II: in 1 of 3 cases with a tumor of the tracheo-bronchial tree of the bronchus of the 1st stage, a complete regression occurred. In the remaining 2 cases – partial regression was observed. 1 Patient with respiratory failure of 2-3 degrees observed a clinical significant partial effect. In one patient after non-radical lung tumor resection, a single session on the stump area prevented tumor relapse for 7 months.
Conclusions: Photodynamic therapy during pulse-mode radiation allows to restore the patency of hollow organs in stenosing cancers of the gastrointestinal tract and bronchus in a short period of time and improves the quality of life. We can observe a radical effect and long-term remission in cases of patients with stage I tumors.

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Discovery and evaluation of a novel phthalazine-based vascular endothelial growth factor receptor 2 inhibitor in glioblastoma cell lines

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Background: Cancer is among the leading causes of mortality, and new targeted therapies protecting off-target cells and tissues ungenrtly needed. Vascular endothelial growth factor receptor 2 (VEGFR-2) inhibitors are among the first clinically approved drug substances for the treatment of tumour-induced angiogenesis. Glioblastoma multiforme (GBM) is one of the most aggressive forms of aggressive brain tumour which is also regulated via vascular endothelial growth factor (VEGF) and its receptor 2 (VEGFR-2). Objectives: (i) to test the hypothesis that in the structurally diverse biochemical space of VEGFR-2 inhibitors with favourable interaction pharmacophores, there is a relationship between chemical structure and activity that can be described by a mathematical model; (ii) to design, screen and in vitro evaluate new active VEGFR-2 inhibitors based only on structural information of the ligands; (iii) to test promising inhibitor(s) in vitro in glioblastoma cell lines.
Materials and methods: Over two hundred VEGFR-2 inhibitors with highly diverse chemical structures were manually collected and curated. Original chemical space was analyzed using machine learning (ML) methodology within the

framework of a quantitative structure-activity relationship (QSAR) approach. To put QSAR into practice, the ZINC15 database was screened with extended scaffolds that were designed to correspond to the application range of the model. The screened structures were ranked based on the QSAR prediction and evaluated against VEGFR-2 enzyme.
Results and conclusion: The resulting QSAR allowed for the analysis of the structure-activity relationship of the compounds. One of several experimentally tested compounds showed high activity at the sub-micromolar level in inhibiting VEGFR-2, with IC50= 0.497±0.04 µM. The anticancer activity of this compound was evaluated against several glioblastoma cell lines. The selected compound was active against H4 and A172 cancer cell lines with EC50=16.3 µM and EC50=23.3 µM, respectively. Its toxicity profile in non-cancerous cells was low. After biological evaluation, it can be concluded that discovered molecule, with unique structural configuration, can be used as lead molecule to design new, potent, and effective VEGFR-2 inhibitors. Further testing of similar molecules in glioblastoma cell lines should be continued.

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P65

Copper(II) complexes of indolobenzazepine-derived ligands with kinase inhibitory potential: mechanism of cytotoxic action in colorectal tumor cells

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Keywords: Antineoplastic agents, copper complexes, indolobenzazepine ligands, colorectal carcinoma hct 116 cells, mtt assay, cell cycle, kinase inhibition

Background: Following the approval of cisplatin (CDDP) as anticancer drug, research into metallotherapeutics has expanded, aiming to develop metal-based drugs with improved efficacy and reduced toxicity. The chemical diversity of metal based complexes enables the development of hybrid drugs with bioactive ligands that may overcome limitations of traditional treatments, exploiting mechanisms beyond DNA binding, such as enzyme inhibition, redox modulation, and interference with cancer signaling. This study explores a new class of ligands structurally related to paullones, which exhibit CDK kinase inhibitory activity. Series of eight novel ligands with an indolobenzazepine core were synthesized and complexed with copper(II) to improve pharmacological properties.
Material and Methods: The biological evaluation *in vitro* included tests for antiproliferative activity, cell cycle effects, kinase inhibition and apoptosis induction, with cisplatin as a reference.
Results and Conclusions: MTT assays in a panel of human tumor cell lines showed that metal-free ligand HL8 and Cu(II) complexes 4 and 8 exhibited the highest cytotoxic activities against colorectal carcinoma HCT 116 cells, with IC50 values in the sub micromolar range (0.8–0.9 µM). A structure-activity relationship analysis suggested that the presence of a methyl substituent at the Schiff base C=N group contributes to the potency of ligand HL8 in HCT 116 cells, an effect maintained in complex 8 (IC50 = 0.8 µM), while in non-tumor MRC-5 cells IC50 = 1.7 µM. Copper coordination also enhanced the activity of HL4 (IC50 =2.03) yielding complex 4 with an improved IC50 of 0.71 µM. Further mechanistic studies including cell cycle analysis revealed that HL4 and complex 4 caused mainly G2/M arrest and increased Sub-G1 apoptotic population. Complex 8 induced a certain Sub-G1 peak, while HL8 led to concentration-dependent cell accumulation in G1 phase (after 24 h), suggesting inhibition of key mediators of G1 phase progression. Kinase inhibition test confirmed strong inhibitory potency of HL8. Annexin V-FITC apoptosis assay revealed the pro-apoptotic effects of complexes 8, HL8, and HL4. Morphological studies showed reduced cell confluency and the presence of rounded, irregular cells in a time- and dose-dependent manner, further supporting apoptosis as the main mechanism of cell death. Indolobenzazepines HL4 and HL8 represent promising structures for further development of metal complexes with multi targeted mode of action.

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P66

Cytotoxic activity of *Thymus lykae Degen & Jav. (Lamiaceae)* essential oil on human cancer cells *in vitro*

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Keywords: cancer cell line, cytotoxicity, essential oil, T. lykae

Background: T. lykae Degen & Jav. is an endemic species from the central Balkans, growing on dry, rocky serpentine hills. Recent research has shown that the essential oil of wild T. lykae possesses antioxidant and antimicrobial activities and could be used as a potential source of natural antioxidants in the food industry. The aim of this study was to determine the cytotoxic potential of the essential oil of T. lykae on certain human cells in vitro: cervical cancer – HeLa, the colon cancer – LS 174, the prostate cancer – PC-3 and the normal fibroblast cells – MRC-5.
Material and Methods: The essential oil was isolated from fresh plant material collected during the flowering period, in Serbia. The air-dried plant parts were cut into small pieces and hydrodistilled for 2 hours using the Clevenger apparatus to extract the essential oil. Cytotoxic activity was determined using the MTT colorimetric assay. Cells were seeded into 96-well microtiter plates and five different concentrations of oil diluted in RPMI-1640 medium were added to the wells. Final dilutions applied to target cells ranged from 250 to 4000 x. The cultures were incubated for 72 h at 37 °C and then 20 µL of MTT solution (5 mg/mL in PBS) was added to wells. Number of viable cells in each well was proportional to the intensity of formazan formed from the conversion of the MTT dye by viable cells.
Results: The results of the cytotoxic activity of the essential oil of T. lykae against cancer and normal cells were determined as IC50 (half maximum inhibitory concentration – as average of dilution factors from three independent experiments). IC50 [dilution] HeLa LS-174 PC-3 MRC-5 1:1998 1:2042 1:2032 1:2155 In all cancer cell lines, the essential oil of T. lykae showed a strong anticancer effect, but the results showed no significant differences between the cancer and normal cell lines.
Conclusions: This study is the first report on the potential antitumor activity of the essential oil of endemic wild T. lykae on cancer cell lines and offers the possibility for further investigations on other cancer cell lines as well.

P67

Study of the anticancer activity of semisynthetic derivatives of sclareol

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Keywords: in vitro anticancer activity, slcareol, semisynthetic derivatives

Background: Sclareol, a natural substance found in many plants, exhibits significant anticancer properties, promoting apoptosis and inhibiting cell proliferation. Purpose: We aimed to study the anticancer activity of semi-synthetic derivatives of sclareol with 1,2,4-triazolo[1,5-a] pyrimidines.
Materials and Methods: 20 derivatives, synthesized and provided by the University of Belgrade (Faculty of Chemistry and Institute for Biological Research "Siniša Stanković" – National Institute of the Republic of Serbia) were tested for their in vitro anticancer activity. The SRB cytotoxicity method was used to study the effect of these substances on the proliferation of four different human cancer cell lines: SF-268 (glioblastoma), NCI-H460 (large cell lung cancer), MCF7 (breast cancer) and HCT-116 (colorectal carcinoma).The substances TNT396 and TNT397, as well as their four

most active derivatives (TNT403, TNT404, TNT405 and TNT406), were studied further for activity against two human pancreatic cancer cell lines: PANC-1 and EANM-ATT021013 (patient-derived cells). Wound healing assay at different concentrations and time intervals was performed to study the ability of these substances to inhibit cell migration. For the ability of the substances to inhibit the formation of colonies by single cells, clonogenic experiments were performed.

Results: 15 out of the 20 substances tested showed strong inhibitory activity (GI50< 6μM). Out of the six substances tested in pancreatic cancer, TNT404 showed the strongest antiproliferative activity against PANC-1 (GI50 = 0.3μM). In the wound healing assay, TNT404 was found to strongly inhibit cell migration in a time- and dose-dependent manner, as well as the formation of PANC-1 colonies.

Conclusions: All 20 semisynthetic derivatives of sclareol with 1,2,4-triazolo[1,5-a]pyrimidines, tested for the first time for their in vitro anticancer activity against six different human cancer cell lines, including a patient-derived PDAC cell population, showed significant anticancer activity. Further research into their mechanism of action is underway in our laboratories.

P68

Exploring the therapeutic potential of *Geum urbanum* in glioblastoma: *in vitro* evaluation on U87 and LN229 cells

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Keywords: anticancer drugs, Geum urbanum, glioblastoma

Background: Glioblastoma represents 49% of malignant brain tumors and is the most common and aggressive type of brain cancer. Current therapy for this malignant brain is surgery, followed by radiotherapy and chemotherapy, and even though its aggressive, prognoses for survival of patients with glioblastoma are poor with high mortality rates. One of the unwanted and limiting features of glioblastoma is resistance to therapy. Finding drugs or molecules that can overcome this restrain is crucial for developing more effective glioblastoma treatments. Natural compounds derived from plants have potential as drugs for the treatment of glioblastoma and are considered as potential anticancer drugs. Geum urbanum belongs to the genus Geum, which is characterized by the presence of bioactive compounds with known anticancer potential. Our aim was to analyze the effect of G. urbanum extracts on glioblastoma cells.

Material and Methods: Four extracts were isolated from G. urbanum and were used for treatment of human glioblastoma cell lines U87 and LN229. 24h after treatment, cytotoxicity of these extracts was studied using MTT assay and microscopy. In addition, we tested the effect of these extracts on U87 sphere viability using Calcein-AM and propidium iodide staining.

Results: Obtained results show that G. urbanum extracts show significant cytotoxic effect on LN229 cell line, while no cytotoxicity was observed on U87 cell line. Microscopic visualization of cells treated with G. urbanum extracts revealed induction of sphere formation in U87 cells, but no morphological changes in LN229 cells. Measurement of sphere viability indicated that G. urbanum extracts had no effect on sphere viability in U87 cells.

Conclusions: Our results indicate that G. urbanum extracts exhibit selective cytotoxic effects on glioblastoma cells, significantly impacting LN229 cells, while demonstrating no cytotoxicity on U87 cells. Interestingly, the extracts promoted sphere formation in U87 cells without affecting their viability, suggesting a possible role in modulating cell behavior rather than inducing cell death in this line. These findings highlight the potential of G. urbanum as a source of bioactive compounds with differential effects on glioblastoma subtypes and support further investigation into its mechanisms of action and therapeutic relevance in glioblastoma treatment.

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Adamantane–sclareol hybrids selectively target multidrug resistant glioblastoma cells by inducing oxidative stress

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Keywords: glioblastoma, innovative anticancer agents, multidrug resistance, oxidative stress, P-glycoprotein

Background: Glioblastoma is an aggressive brain malignancy characterized by a limited therapeutic response, primarily due to multidrug resistance (MDR). One critical contributor to MDR is the activity of P-glycoprotein (P-gp), an efflux transporter that decreases drug accumulation within cells. Inhibiting P-gp and disrupting redox homeostasis may present viable strategies to counteract MDR. This study evaluates the in vitro biological activity of two adamantane-sclareol hybrid compounds (1 and 2) in terms of glioblastoma cell viability, P-gp functionality, and oxidative stress.
Materials and Methods: Our experimental model comprises the human glioblastoma U87 cell line (P-gp-negative), its drug-resistant counterpart U87-TxR (P-gp-positive), and normal human astroglial SVG p12 cells. Cytotoxicity assays for compounds 1 and 2, alone or in combination with paclitaxel (PTX), were conducted via the MTT. Flow cytometry was employed to assess P-gp activity, apoptosis induction, and intracellular reactive oxygen species (ROS) levels. Real-time PCR was performed to quantify the expression of antioxidant-related genes.
Results: Compounds 1 and 2 exhibited a more pronounced growth inhibition in glioblastoma cells compared to normal SVG p12 cells. These hybrids bypassed MDR in glioblastoma cells, illustrated by lower IC50 values in MDR U87-TxR cells relative to sensitive U87 cells. Apoptosis rates were significantly higher in U87-TxR cells when treated with the hybrids compared to U87 cells. Furthermore, both compounds enhanced the intracellular accumulation of the P-gp substrate, rhodamine 123, indicating their P-gp inhibitory effects. The combination of PTX with compounds 1 and 2 demonstrated predominantly synergistic interactions. Notably, both hybrids increased intracellular ROS levels, especially in U87-TxR cells. This oxidative stress response was also followed by upregulation of antioxidant-related genes in both glioblastoma lines.
Conclusions: The adamantane-sclareol hybrids exhibit selective cytotoxicity against glioblastoma cells, with a marked effect on MDR glioblastoma cells, as confirmed by MTT assay. This selective cytotoxicity was also confirmed by enhanced apoptosis induction and disruption of the redox balance, indicating a phenomenon known as collateral sensitivity. Additionally, both hybrids inhibited P-gp activity and displayed synergistic interactions with PTX, underscoring their therapeutic potential in the treatment of glioblastoma.

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The effects of novel diclofenac-based carborane-substituted prodrug on colon cancer cell lines and mouse-derived organoids

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Keywords: carboranes, colon cancer, diclofenac, drug repurposing, non-steroidal anti-inflammatory drug (NSAID), organoids

Background: Drug repurposing is a promising strategy in drug development in oncology. A notable example of this approach is diclofenac, a nonsteroidal anti-inflammatory drug (NSAID) traditionally used for pain relief, which has shown anticancer potential via COX-dependent and COX-independent mechanisms. In this study, a novel anticancer compound, a unique carborane derivative of diclofenac with a zwitterionic amidine structure and a nido-carborane cluster, was developed using fragment-based drug discovery. Designed as an engineered prodrug, it aims to overcome traditional NSAID limitations and enhance biological effects.
Material and Methods: The anticancer properties of the compound were tested against three cancer cell lines: murine colon adenocarcinoma (MC38), human colorectal carcinoma (HCT116), and human colorectal adenocarcinoma (HT29), as well as peritoneal exudate cells (PECs) isolated from healthy mice and on the human fetal lung fibroblast cell line MRC-5. Furthermore, the effects of the experimental compound were tested on colon cancer mouse-derived organoids. Cell viability was measured using MTT and CV assays on cell lines and the CellTiter-Glo® assay on organoids. The mechanism of action of the substance on the MC38 cell culture was evaluated by flow cytometry and fluorescence microscopy.
Results and Conclusions: The tested compound showed strong cytotoxicity on all cancer lines after 72 h, with IC50 values for PECs and MRC-5 being 2–3 times higher, indicating moderate selectivity towards malignant cells. Treatment also decreased the viability of organoids. Flow cytometry analysis revealed that the cytotoxicity of the substance is based on caspase-independent apoptosis preceded by cell division blockage. Fluorescence microscopy after PI staining confirmed apoptosis, cells with possibly undivided genetic material, and senescence cells. Acridine orange staining showed increased presence of autophagosomes, but co-treatment with autophagy inhibitors further reduced viability, suggesting a pro-survival role of autophagy. The production of reactive oxygen and nitrogen species (ROS/RNS) in the presence of the tested compound was strongly reduced, and it was in concordance with the efficient inhibition of COX-2. Our findings suggest that carborane-based prodrugs offer a promising route for developing new anticancer therapies, especially as adjuvant or combination therapies. The presented results open valuable possibilities for further research.

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Novel aroylacrylic acid phenylamides revealed strong prooxidative activity and consequent cell death induction in A549 (KRASmut) and Mcf7 (KRASwt) cancer cell lines

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Keywords: aroylacrylic acid phenylamides, reactive oxygen species, mitochondria, KRAS

Background: This study was organized to unravel the mechanism responsible for induction of cell death by treatment with aroylacrylic acid phenylamides. Four novel structural analogues were designed (1a-1d) by means of Michaels' adduction of seconadary cyclic amines to aroylacrylic acid phenylamides. A549 and Mcf7 cell lines were used as being KRASmut and KRASwt, respectively. The difference in their KRAS status implies that A549 and Mcf7 cells utilize disparate metabolic strategies to produce energy, and exhibit different responses to applied treatments.
Material and Methods: Cell death was determined by Annexin V/propidium iodide (PI) staining, mitochondrial superoxide anions (MtSR) were detected by MitoSOX Red, total reactive oxygen species (ROS) by DCFDA. Morphological integrity of mitochondria and mitochondrial network was determined by MitoTracker CMX Ros (MTR) staining. Analyses were performed by flow cytometry and fluorescent microscopy.
Results: Results of the Annexin V/PI staining after 24 h of treatment showed that Mcf7 cells were more sensitive than A549 cells. Cell death response was reversed by co-incubation with N-acetylcysteine (NAC) in both cell lines confirming that ROS generation was the mechanism of compounds' activity. At lower time-frame (6 h), significantly higher magnitude of cumulative MtSR production was found only in Mcf7 cells treated with 1a, whereas dimmed

MtSR-positive subpopulation was dominant in all treated A549 cells. The true degree of ROS overburden in Mcf7 cells was revealed by a massive increase in DCFDA-positive events. MTR staining of A549 cells showed the simultaneous presence of hypo and hyperpolarized mitochondria within the same cell, indicating a variety in cellular mitochondrial polarization, whereas integrity of mitochondrial network was severely affected in Mcf7 cells.
Conclusions: Increased survival of A549 and Mcf7 cells co-incubated with 1a-1d and NAC served as a validation that both cell lines were affected by ROS generation upon treatment. Dysfunctional oxidative phosphorylation (OXOPHOS) in KRASmut A549 cells indicated that ROS generation was not launched at the level of mitochondrial respiration complexes I-V. Mcf7 cells were more susceptible to experimental treatments than A549 cells at both 24 h and 6 h of investigation. These results imply that fully operative OXOPHOS in KRASwt cells exceedingly amplifies ROS production in response to investigated compounds.

P72

Dual diagnostic and therapeutic use of photoactivable BODIPY dyes in cancer

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Keywords: anticancer phototherapy, BODIPY, protein labelling, theranostics

Background: Quinone methides (QMs) are reactive intermediates derived from phenols, capable of alkylating and cross-linking DNA, and are linked to the anticancer activity of drugs like mitomycin and anthracyclines. Their targeted photochemical generation in tumors presents a promising strategy for novel phototherapeutics. We have developed several QM precursors and explored their photocytotoxicity. Recently, we focused on QM precursors linked to BODIPY chromophores, aiming to activate them using visible light. Notably, we observed unusual photoreactivity from higher excited states, enabling fluorescent photolabeling of proteins. To advance potential theranostic applications, it is essential to evaluate the subcellular localization, photoinduced cytotoxicity, and protein-labeling capabilities of these compounds.
Materials and Methods: The antiproliferative activity of BODIPY–QM compounds was assessed using the MTT assay on various human and mouse cancer and non-cancer cell lines. Intracellular localization was analyzed by confocal microscopy. Photolabeling experiments were conducted by irradiating model proteins (e.g., bovine serum albumin, human serum albumin, and dipeptidyl peptidase III – DPP III), followed by SDS-PAGE. The enzymatic activity of irradiated DPP III was also evaluated.
Results: In the absence of light, the compounds exhibited little or no cytotoxicity. However, upon visible-light irradiation, several compounds showed marked cytotoxicity, confirming photoactivation-dependent effects. Confocal imaging revealed that BODIPY derivatives localize predominantly in the cytoplasm and membrane-rich organelles, as confirmed using organelle-specific antibodies. Irradiation of model proteins led to covalent dye–protein binding, with efficiency varying between proteins. Optimal concentrations and irradiation times were determined for effective photolabeling.
Conclusions: BODIPY-based QM precursors show strong potential for the development of photoactivatable anticancer agents, effective under both normoxic and hypoxic conditions. Furthermore, their capacity for fluorescent protein labeling supports their use in innovative diagnostic or theranostic approaches.

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In vitro antitumor activity of novel Schiff base-derived palladium complexes

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Keywords: drug design, biogenic amines, cancer of prostate, palladium, apoptosis, molecular docking simulation

Background: The treatment of cancer represents the most challenging issue of modern science, particularly due to the resistance of tumor cells to many utilized protocols and treatments, as well as serious side effects. Thus, the development of new anti-cancer agents with increased activity and lowered toxicity is one of the major directions of modern science. Due to structural resemblance with well-known platinum compounds, palladium-based metallo-drugs have found a significant place in cancer research. In this study, we aimed to determine the cytotoxic effect of three novel synthesized Pd(II) complexes with Schiff bases as ligands, on the PC-3 and DU-145 cell lines, along with healthy fibroblast cell line MRC-5.
Materials and methods: Cells were treated with ligands and palladium complexes and cell viability and death induction upon treatment were examined.The molecular docking studies were performed to support experimentally obtained results. In addition, the fluorometric quenching assay was performed to determine the binding mode between tested complexes and the DNA molecule.
Results: All screened complexes showed high cytotoxic activity toward selected cell lines, while complex **2c** combining salicylic and tryptamine structural motifs, demonstrated even better activity than cisplatin for both tested cell lines, with IC_{50} values of 7.1 µM (DU-145) and 8.6 µM (PC-3). Flow cytometry analysis showed that all complexes are capable of inducing apoptosis through the Bcl-2 and caspase family activation. Sophisticated molecular docking studies supported the experimental findings, while fluorescence assay indicated that complexes interact with double-helical DNA through both binding modes, the intercalation and minor groove binding.
Conclusions: The findings arising from this study have underscore the selective anticancer potential of the newly synthesized Pd(II) complexes (**2a–c**), particularly their preferential activity against human prostate cancer cell lines. Taking into consideration the fact thatphysico-chemical and pharmacological properties of Schiff bases can be easily tuned through structural modification, these results offer numerous possibilities for further manipulation of ligand and complex structure in order to find more effective, more selective and less toxic anti-cancer metallo-drug candidates.

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Comparative Transcriptome Analysis of Caspase and Apoptosis Pathway of K562 cells treated with butin, butein, and sulfuretin from the heartwood of Cotinus coggygria Scop.

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Keywords: Apoptosis

Background: This research examined the anticancer effects of butin, butein, and sulfuretin, which are derived from the heartwood of Cotinus coggygria, against K562 cells. In K562, the main treatment approach focuses on targeting BCR-ABL signaling with TKIs. To explore apoptosis induction as an additional therapeutic approach, the study aimed to determine whether these natural compounds can enhance or restore apoptotic responses in p53-null leukemia cells.
Material and Methods: The study employed flow cytometry to assess the patterns of apoptosis and caspase activation. RNA-Seq was used to substantiate the results obtained from the cellular assay.
Results: In the caspase inhibitor assays, each compound exhibited a distinct dependency profile (butin and butein on caspase-3/-8, and sulfuretin primarily on caspase-3), and the RNA-Seq results provided a matching molecular "fingerprint" for these differences. Notably, butin and sulfuretin both induced transcriptional upregulation of CASP7 and CASP10, whereas butein did not significantly alter these particular caspases. Conversely, butein and sulfuretin upregulated CASP9 (mitochondrial pathway). Only sulfuretin led to a significant downregulation of CASP8 transcripts, consistent with its reduced reliance on the extrinsic initiator caspase. All three treatments induced the upregulation of pro-apoptotic genes, including BAX, BAK1, and APAF1, as well as FADD, TRAIL, and TNFRSF10B, indicating a pro-apoptotic transcriptional program. Butein, which influenced the highest number of genes (809 DEGs), triggered both the extrinsic and intrinsic pathways and exhibited the most extensive enrichment of apoptosis-related genes. Butin predominantly engaged the extrinsic pathway with a strong executioner caspase response, while showing minimal impact on intrinsic caspase-9, aligning with its caspase-8 dependence. Sulfuretin, with the fewest DEGs (299), still upregulated CASP9 and CASP7 but uniquely repressed CASP8, implying that it may bypass the extrinsic route. Sulfuretin's transcriptome showed strong downregulation of anti-apoptotic factors (e.g., BCL-2 family and IAP genes).
Conclusions: All three investigated compounds, flavanone butin, chalcone butein, and aurone sulfuretin, converge on the induction of apoptosis, as evidenced by both functional assays and GSEA data.

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DPP3 knockdown in HeLa cells induces G1 arrest

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Keywords: DPP3, CDKN1A, Cell Cycle, Flow Cytometry, HeLa cells, RNA Interference

Background: Dipeptidyl peptidase 3 (DPP3) is a cytosolic zinc-dependent metallopeptidase that contributes to the intracellular degradation of peptides and the regulation of oxidative stress. DPP3 is overexpressed in a variety of cancers (endometrial, ovarian, lung, breast, colorectal and esophageal) suggesting its possible role in cancer development.

Because of the interplay between stress signaling and cell cycle checkpoints, the objective of our research was to investigate DPP3 knockdown effects on cell cycle regulatory pathways in cervical carcinoma cells, so we analysed cell cycle distribution and the expression of CDKN1A (p21), a cyclin-dependent kinase inhibitor, that is related to cell cycle arrest and is typically elevated due to cellular stress, after DPP3 knock-down in HeLa cells.
Material and Methods: HeLa cells were subjected to DPP3 depletion by siRNA-mediated knockdown. At 48 hours post-transfection, cells were harvested for protein isolation and subjected to SDS-PAGE and western blot analysis to assess knockdown efficiency and p21 protein levels, alongside mRNA quantification by qPCR. For cell cycle analysis, cells were ethanol fixed, treated with RNase, and stained with propidium iodide. DNA content was analyzed by flow cytometry to determine cell cycle phase distribution.
Results: DPP3 silencing in HeLa cells resulted in effective knockdown as confirmed by western blot, accompanied by an increase in CDKN1 (p21) expression at both mRNA and protein levels. Flow cytometry analysis demonstrated significant G1 phase arrest, indicating an inhibitory effect on cell cycle progression.
Conclusions: Our results indicated that DPP3 knockdown results in G1 phase arrest in HeLa cells by increasing p21 expression, supporting DPP3 as a potential negative regulator of the cell cycle, in relation to a p21-mediated pathways, and a potential therapeutic target for future cancer therapies.

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Sirtuin 3 enhances stress sensitivity and cell cycle disruption in triple-negative breast cancer

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Keywords: Sirtuin 3, MDA-MB-231, breast cancer, DNA damage, cell cycle

Background: Triple-negative breast cancer (TNBC), an aggressive subtype lacking estrogen receptor, progesterone receptor, and HER2 expression, is associated with high recurrence rates and limited targeted treatment options. Sirtuin 3 (Sirt3) is a mitochondrial NAD^+-dependent deacetylase that plays a central role in regulating cellular metabolism, oxidative stress, and mitochondrial function. In breast cancer, Sirt3 has been suggested to have a potential dual role, acting either as a tumor suppressor or as a promoter of cancer progression. We previously demonstrated that overexpression of Sirt3 diminishes tumorigenic properties in the TNBC cell line MDA-MB-231, supporting its potential tumor-suppressive function in this context. In the present study, we further investigated the effects of Sirt3 overexpression in MDA-MB-231 cells under normal and hyperoxic conditions.
Materials and methods: We use MDA-MB-231 breast cancer cells transfected with the FLAG-tagged Sirt3 (MDA-S3) or empty pcDNA3.1 plasmid (MDA-C). Upon hyperoxic treatment, cells were analyzed for metabolic activity using MTT assay. Apoptosis and cell cycle distribution are quantified via flow cytometry. Cell morphology and subcellular localization of proteins of interest, as well as DNA damage were analyzed using confocal microscopy. Western blot was used to analyze or confirm protein expression.
Results: Our findings reveal that Sirt3 increases metabolic activity in MDA-MB-231 cells while simultaneously promoting apoptosis and DNA damage. Notably, exposure to hyperoxic conditions further amplified these effects, resulting in enhanced apoptosis, higher levels of DNA damage, and cell cycle disruption characterized by S-phase arrest. These results indicate that, despite promoting metabolic activity, Sirt3 sensitizes TNBC cells to hyperoxic stress, ultimately enhancing cell death.
Conclusions: Taken together, these data expand on previous observations by demonstrating that Sirt3 not only diminishes tumorigenic potential but also modulates stress susceptibility, genome stability, and cell cycle progression in TNBC cells. This supports the hypothesis that Sirt3 functions as a tumor suppressor in TNBC and highlights its potential as a target for therapeutic strategies aimed at increasing vulnerability of cancer cells to metabolic and oxidative stress.

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Cytotoxic evaluation of novel 4-oxothiazolidine derivatives on human cancer cell lines

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Keywords: 4-oxothiazolidine derivatives, cell survival, neoplasms

Background: 4-oxothiazolidine and its derivatives are an important class of heterocyclic compounds known for their diverse pharmacological effects, including anticancer activity. Their low cost of synthesis, structural diversity, and potential for selective cytotoxicity make them valuable candidates for further research. This study aimed to investigate the in vitro cytotoxic activity of selected 4-oxothiazolidine derivatives on various cell lines.
Material and Methods: The cytotoxicity of 14 synthesized 4oxothiazolidine derivatives, divided into three groups ((1) compounds 1-6, no substituent; (2) compounds 7-10 with 2ethoxy2oxoethyl substituent; and (3) compounds 11-14, methyl substituent) based on the substituent at position 5 of the thiazolidine ring, was assessed using the Sulforhodamine B assay on 6 human cell lines: HeLa, HCT-116, MIA PaCa-2, PANC-1, MDA-468 cancer cells, and MRC-5 normal lung fibroblasts, 72 h after treatment.
Results: All tested compounds exhibited varying cell line-specific effects, with significant differences in both activity and selectivity. The HCT116 cells showed an excellent response to treatment with all analyzed 4-oxothiazolidine derivatives, with viability percentages 14.3-46.5%, except for compound 1 (73.5%). HeLa cells were particularly sensitive to compounds 4 and 6, with approximately 10% of viable cells remaining, while they showed moderate sensitivity to the other compounds (30-60%). Compounds 6 and 9-14 exhibited significant cytotoxic effects, reducing the viability of MIA PaCa-2 cells to 23.5-56.1%, while all other compounds had little to no effect (70.7-92.7%). PANC-1 and MDA-468 cells demonstrated considerable resistance to 4-oxothiazolidine derivatives, with viability of over 58% and 59.6%, respectively. Notably, MDA-468 cells showed stimulation after treatment with compounds 1 and 6. Compound 9 exhibited the best selectivity, with 73% of fibroblasts remaining viable post- treatment.
Conclusions: The tested 4-oxothiazolidine derivatives showed cell-specific cytotoxic effects, with significant variability in both potency and selectivity among the derivatives. Some cancer cell lines exhibited greater sensitivity to these compounds, suggesting that their effectiveness may vary depending on specific tumor types or cellular characteristics. In contrast, other cell lines showed moderate to low sensitivity, highlighting the importance of understanding how these compounds interact with different cancer contexts.

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Two novel pyrimidine-artesunate hybrids overcome multidrug-resistance in non-small cell lung carcinoma through collateral sensitivity

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Keywords: artesunate, collateral sensitivity, multidrug-resistance, non-small cell lung carcinoma, P-glycoprotein, pyrimidine

Background: Two novel hybrid compounds (1 and 2) based on artemisinin and pyrimidine were tested for their anticancer activity against non-small cell lung carcinoma (NSCLC), specifically targeting multidrug-resistance mediated by P-glycoprotein (P-gp).
Materials and Methods: We used the human NSCLC cell line NCI-H460 (without P-gp expression), its multidrug-resistant counterpart NCI-H460/R (with P-gp expression), and the normal human lung fibroblast cell line MRC-5. Cell viability after treatment with 1 and 2, alone or in combination with the P-gp inhibitors dexverapamil (Dex-Ver) and tariquidar (TQ), was assessed using the MTT assay. Accumulation of the rhodamine 123 (Rho 123), a P-gp substrate, was analyzed by flow cytometry.
Results: Compounds 1 and 2 exhibited enhanced cytotoxicity towards resistant NCI-H460/R cells, indicating collateral sensitivity. Both compounds also showed significant selectivity for cancer cells. Flow cytometry analysis revealed that 1 and 2 effectively inhibited P-gp activity, as evidenced by the increased accumulation of Rho 123. Simultaneous treatment of 1 and 2 with P-gp inhibitors indicated that the type of P-gp inhibition matters. The substrate inhibitor Dex-Ver lowered cytotoxicity of 1 and 2, while the non-substrate inhibitor TQ, increased it. Flow cytometry also showed that co-treatment with Dex-Ver further increased Rho 123 levels, indicating additive inhibition. However, when combined with TQ, Rho 123 accumulation was comparable to that of TQ alone, suggesting that P-gp was already maximally inhibited and the compounds made no further contribution.
Conclusions: The study demonstrates that pyrimidine-artesunate hybrids selectively target MDR cancer cells, emphasising their potential to overcome drug resistance in cancer treatment.

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Investigating the *In Vitro* Cytotoxicity of Novel Pyrido-dipyrimidine Compounds

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Keywords: Apoptosis, Caspases, Cytotoxicity

Background: Pyrido-dipyrimidine compounds have gained substantial attention in medicinal chemistry due to their potential as anticancer agents. These compounds are known to inhibit essential pathways involved in tumor growth and proliferation, positioning them as promising therapeutic candidates. The present study investigates the in vitro

cytotoxic effects of a newly synthesized class of pyrido-dipyrimidine compounds against human cancer cell lines, aiming to evaluate their therapeutic potential and underlying mechanisms of action.
Material and methods: To evaluate the cytotoxic effects, 25 pyrido-dipyrimidine compounds (C1-C25) were tested on human cell lines: HeLa (cervical adenocarcinoma), K562 (chronic myeloid leukemia), LS174 (colon adenocarcinoma), A549 (lung adenocarcinoma), and MRC5 (non-malignant fetal lung fibroblasts). Cells were exposed to graded concentrations of the compounds and incubated for 24, 48, and 72 hours, followed by assessment of cell survival via the MTT assay. The most active compounds were selected for further investigation, specifically targeting HeLa and K562 cell lines. Flow cytometry (propidium iodide staining) and fluorescence microscopy (acridine orange/ethidium bromide staining) were utilized to analyze cell cycle distribution and morphological changes. Additionally, selective caspase inhibitors were used to elucidate the apoptotic pathways involved.
Results: Results showed that the compounds exhibited concentration-dependent cytotoxicity. HeLa and K562 cells demonstrated the highest sensitivity, with C17 and C9 yielding the lowest IC50 values (9.82±1.07 µM and 17.36±1.39 µM, respectively) and displaying high selectivity over MRC5 cells (selectivity coefficients of 12.46 for C17 and 9.20 for C9). Further analysis revealed that these compounds significantly increased the SubG1 cell cycle phase, suggesting apoptosis induction. Fluorescence microscopy with AO/EB staining further confirmed characteristic apoptotic morphological changes. Flow cytometric analysis indicated that apoptosis induced by C17, was inhibited by caspase inhibitors, implicating caspase-3, -8, and -9 in its apoptotic pathway, while C9 functioned via a caspase-independent mechanism.
Conclusions: These findings suggest that novel pyrido-dipyrimidine compounds C17 and C9 are potent, selective cytotoxic agents with distinct apoptotic mechanisms, highlighting their potential as candidates for future cancer research.

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miR-219: A Novel Radiosensitizing Candidate in Glioblastoma Treatment

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Keywords: radiotherapy, GBM, miRNAs, radiosensitization

Background: Glioblastoma multiforme (GBM) is the most prevalent and aggressive form of malignant brain cancer in adults, with a 5-year overall survival rate of 9.8%. It is characterized by rapid growth and a highly invasive nature. The standard approach for GBM treatment includes surgical resection followed by radiotherapy combined with temozolomide therapy. Improving the response of GBM cells to radiotherapy remains one of the major challenges in clinical oncology, and discovering novel molecules that can enhance radiosensitivity is a promising strategy to overcome this hurdle. MicroRNAs (miRNAs) regulate key processes in GBM progression and can influence radiosensitivity by targeting radiation-related pathways, making them promising therapeutic targets. The brain-specific miRNA miR-219 is already recognized as an important tumor suppressor in GBM. The main aim of this study is to analyze how modulating miR-219 expression affects the radiosensitivity of GBM cells.
Methods: GBM cell line LN229 was transduced with a miR-219 lentiviral construct for overexpression or with a control vector. Transduced cells were then seeded into different dishes and, the next day, irradiated with 4 Gy or sham-irradiated. To analyze the migration potential, a wound scratch assay was performed. The sphere-forming ability was assessed using a sphere formation assay, including measurements of sphere size and propidium iodide (PI) staining. Expression of the Vimentin marker, associated with epithelial-to-mesenchymal transition and GBM stem cells, was analyzed in both 2D and 3D cultures using immunocytochemistry.
Results: LN229 cells overexpressing miR-219 showed a significantly lower migration rate compared to control cells under both sham-irradiated and irradiated conditions. Spheroids formed by miR-219 overexpressing cells exhibited a significantly lower growth rate compared to control cells. PI staining confirmed a higher proportion of dead cells in both sham-irradiated and irradiated conditions, associated with miR-219 overexpression. Also, Vimentin expression was reduced in cells with miR-219 overexpression in both 2D and 3D models, under sham-irradiated and irradiated

conditions.
Conclusions: miR-219 overexpression reduces migration, growth, and Vimentin expression in GBM cells, while increasing cell death under both sham and irradiated conditions. These results suggest that miR-219 may enhance radiosensitivity and could serve as a potential therapeutic target in GBM.

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Teucrium extracts as promising sources of anticancer agents and bioactive phytochemicals

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Keywords: Key words: cytotoxicity, cell cycle, anticancer activity, plant extracts

Background: Traditional medicine indicates that plants of the genus Teucrium are rich in polyphenols, which are of interest due to their potential anticancer properties. In this study, we focused on evaluating the cytotoxic activity of two ethanolic extracts (T. montanum & T. capitatum), as well as their effects on cell cycle distribution. The plant material was collected in Montenegro.
Material and methods: The cytotoxic activity (IC_{50}) of Teucrium extracts was evaluated on six human cell lines: HeLa (cervical adenocarcinoma), MDA-MB-231 (breast adenocarcinoma), A549 (lung carcinoma), A375 (malignant melanoma), LS 174T (colorectal adenocarcinoma), and HaCaT (normal keratinocytes). The effects of the extracts on the distribution of HeLa cells throughout the cell cycle were assessed by flow cytometry. Phytochemical composition (total phenolics, flavonoids, anthocyanins) was quantified spectrophotometrically, while mineral content was measured using ICP-MS and ICP-OES.
Results: Teucrium extracts had high concentrations of polyphenolic compounds. In terms of mineral content, the samples were very rich in essential minerals, with T. montanum showing notably high levels of phosphorus, magnesium and iron. These results highlight the nutritional and bioactive potential of both Teucrium species. Both extracts exhibited low cytotoxic activity against cancer cell lines, with the highest intensity observed on HeLa cells (IC_{50} = 256.33 ± 10.31 µM for T. capitatum and IC_{50} = 314.14 ± 20.83 µM for T. montanum). T. capitatum also showed activity against A549 (IC_{50} = 238.37 ± 43.71 µM) and A375 (IC_{50} = 224.99 ± 5.19 µM) cells, whereas T. montanum displayed lower cytotoxicity against tested lines. LS 174T and MDA-MB-231 cells were resistant to both extracts. These results indicate that both Teucrium species contain bioactive compounds warranting further studies on their anticancer mechanisms. Compared to the control, T. montanum & T. capitatum increased the percentage of cells in subG1 phase and G2/M phases of the cell cycle after 24 h and 48 h treatment.
Conclusion: The ethanolic extracts of T. capitatum and T. montanum exerted cytotoxic effects against various human cancer cell lines and induced cell death of HeLa cells. They were also found to be rich in polyphenolic compounds, highlighting their bioactive potential. Both species warrant further research to understand their anticancer effects.

SESSION 12

TOXIC TRIGGERS: ENVIROMENTAL CHEMICALS AND CANCER RISK

P82

Protective Role of Mangiferin Against Bisphenol A-Induced Toxicity

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Keywords: Bisphenol A, Mangiferin, Protective effect, Endocrine-disruptor, HUVEC

Background: Bisphenol A (BPA), a widely used industrial compound, is recognized for its endocrine-disrupting potential and its detrimental effects on multiple organ systems, including the reproductive, neurological, hepatic, and cardiovascular systems. Chronic exposure to BPA, even at low doses, has been associated with oxidative stress, inflammation, and metabolic dysfunction. Mangiferin, a natural xanthone glycoside primarily isolated from Mangifera indica, has demonstrated potent antioxidant, anti-inflammatory, and cytoprotective properties in various experimental models. This study aims to evaluate the protective effect of mangiferin against BPA-induced cellular and systemic toxicity in vitro.
Methods: The cytotoxic effect of siramesine on endothelial cells (HUVEC) was examined with the SRB. HUVEC cells were exposed to Bisphenol A (BPA) at concentrations ranging from 10 to 100 µM. This exposure was conducted in the presence or absence of Mangiferin, a compound that was administered at concentrations ranging from 10 to 100 µM. Human Umbilical Vein Endothelial Cell (HUVEC) cells were exposed to bisphenol A (BPA) at concentrations of 5, 45 and 90 µM, 5 µM MNG, and a combination of both, over a 24-48 hour period. This experimental approach was adopted for cell cycle analysis and wound healing assay. The primary objective of this investigation was to ascertain the protective effect of MNG.
Results: Mangiferin demonstrated no cytotoxic effect on HUVEC cells within the applied dose range. The investigation revealed that the presence of bisphenol A resulted in a dose-dependent enhancement in the degree of cytotoxic effect, within the established range of doses. The Bisphenol A GI50 dose was determined to be 88.01 µM, the TGI dose was 134.34 µM, and the LC50 dose was 179.5 µM. Cell viability increased in the combination of Bisphenol A 90 µM and Mangiferin 5 µM. The results of the cell cycle and wound healing experiments provide support for cell viability results.
Conclusion: Mangiferin is hypothesized to attenuate BPA-induced cell stress, normalize cell behavior, and preserve cell integrity. Our results will provide critical insights into the protective efficacy of mangiferin against BPA toxicity and contribute to the development of plant-derived interventions for environmental toxicant exposure.

SESSION 13

TUMOR METABOLISM

P83

Sodium bicarbonate–induced alkalization modulates tumor cell viability and metabolic activity in vitro

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Keywords: alkalizing therapy, tumor cells, sodium bicarbonate

Background: Tumor cells display a hyperactive metabolism marked by aerobic glycolysis (the Warburg effect), leading to excess lactate production and extracellular acidosis, which promotes invasion, metastasis, and extracellular matrix remodeling – hallmarks of the acidic tumor microenvironment that enhance cancer cell survival and malignancy. This study aimed to investigate the effects of alkalizing tumor cells in vitro using sodium bicarbonate (NaHCO3) as a pH-modulating agent.
Materials and Methods: Mouse CT26 colon adenocarcinoma and patient-derived melanoma cells were cultured at 37°C, 5% CO_2 in RPMI-1640 or DMEM/F12 medium supplemented with 10% fetal bovine serum, and 50 µg/mL gentamicin. Alkalization was induced by supplementing the culture media with NaHCO3 solution, adjusted for baseline bicarbonate concentrations in each medium. Medium pH was monitored via phenol red spectral changes, metabolites were analyzed biochemically, and apoptosis (Annexin V-FITC/PI), lysosomal activity (Lyso Green), proliferation (Ki67), and cell migration and monolayer recovery (scratch wound assay) were assessed according to manufacturer's instructions.
Results: Elevated $NaHCO_3$ concentrations significantly reduced cell proliferation and increased cytotoxicity. No further increase in cell number was observed at $NaHCO_3$ concentrations exceeding 0.95 M. While $NaHCO_3$ supplementation proportionally raised the initial extracellular pH, progressive acidification occurred over time due to ongoing cellular metabolism and metabolite accumulation, despite regular medium changes. Concomitant decreases in glucose and glutamine levels, along with increased lactate production, indicated sustained metabolic activity. At higher $NaHCO_3$ concentrations, glucose was completely depleted within 48 hours without medium renewal. Additionally, elevated bicarbonate levels enhanced lysosomal activity, as detected by flow cytometry using Lyso Green, and delayed monolayer wound healing in both CT26 and melanoma cells.
Conclusions: Alkalizing tumor cells with sodium bicarbonate may exert antitumor effects by disrupting metabolic homeostasis and inducing programmed cell death. These findings suggest that modulating the acidic tumor microenvironment through bicarbonate-based interventions could represent a promising adjunctive strategy in cancer therapy.

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Overcoming oxygen heterogeneity in cancer photodynamic therapy to boost cytotoxicity

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Keywords: cancer, control of oxygenation, photodynamic therapy, pulse-mode irradiation

Background: Photodynamic therapy (PDT) represents an important addition to traditional cancer treatment methods. However, tumor hypoxia and oxygen heterogeneity significantly limit its clinical effectiveness. The development of strategies to overcome these limitations is a pressing issue. The aim of this study was to evaluate the impact of metabolic oxygen consumption in tumor spheroids formed from mouse CT26 colon adenocarcinoma cells on the formation of hypoxic zones and the efficacy of photodynamic action (PDA). **Materials and Methods:** Tumor spheroids were formed by seeding CT26 cells into low-adhesion round-bottom plates. A chlorin e6-based photosensitizer was used, and irradiation was performed using a 662 nm laser. Modeling of oxygen concentration changes within 650 µm diameter spheroids was carried out using COMSOL Multiphysics, taking into account cellular metabolic oxygen consumption. Assessment of photochemical oxygen consumption was conducted using a macroscopic model of singlet oxygen generation. **Results:** It was shown that cellular metabolic activity leads to the formation of an oxygen gradient and the establishment of a hypoxic zone (with oxygen concentration below 1 µM) in the center of the spheroid. Spheroids were classified by size: small (100–400 µm), medium (400–650 µm), and large (650–1200 µm). Hypoxic tumor zones demonstrated protection against PDA at an irradiation dose of 15 J/cm², which affected cytotoxic efficacy and promoted spheroid regrowth. Medium-sized spheroids exhibited the highest resistance. Growth suppression in large spheroids was achieved after PDA with a dose of 15 J/cm² and an average power density of 12.5 mW/cm². Mathematical modeling showed that metabolic oxygen consumption reduces oxygen concentration at the spheroid surface to 70–80 µM, forming hypoxic zones in the center. During PDA, an increased oxygen consumption rate and reduced surface oxygen concentration were observed. The viable cell zone protected from PDA was located at the boundary adjacent to the necrotic core, where oxygen concentration ranged from 1 to 10 µM. **Conclusions:** To enhance PDT efficacy, it is crucial to account for tumor oxygen levels as low as 1–10 µM, caused by high metabolic consumption and limited diffusion. Computational modeling of singlet oxygen generation supports experimental findings and underscores the need for optimized irradiation protocols to overcome hypoxia and improve treatment outcomes.

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Targeting glycolysis with 2-Deoxy-D-Glucose and lysosomal integrity with L-Leucyl-L-Leucine methyl ester as antimelanoma strategy

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Keywords: lysosomes, cathepsins, melanoma, glycolysis, energy metabolism

Background: Malignant melanomas are characterized by enhanced glycolysis, which supplies energy and biosynthetic

precursors for rapid proliferation. Melanomas also exhibit increased lysosomal fragility and elevated levels of lysosomal enzymes cathepsins. Cathepsins promote metastasis and immune evasion, but when released into the cytosol upon lysosomal membrane permeabilization, they trigger cell death. This study aimed to evaluate the antimelanoma potential of 2-deoxy-D-glucose (2DG), an inhibitor of the glycolytic enzyme hexokinase, in combination with L-leucyl-L-leucine methyl ester (LLOMe), a lysosome-destabilizing agent.

Materials and Methods: Gene expression data from melanoma patients and normal skin (GEO dataset GSE3189) were analyzed using the Mann-Whitney U test. Human A375 melanoma cells were treated with 2DG, LLOMe, or their combination. Cell viability was assessed by MTT assay. Flow cytometry was used to assess apoptosis by detecting phosphatidylserine externalization (Annexin V/PI staining) and DNA fragmentation (sub-G1 DNA content analysis of PI-stained cells). Caspase activation was measured fluorometrically (ApoStat staining). The type of 2DG+LLOMe-induced cell death was determined by assessing viability of cells pretreated with inhibitors of caspases, necroptosis, ferroptosis, and autophagy.

Results: GEO analysis revealed elevated expression of glycolytic enzymes (hexokinase, phosphoglucose isomerase, platelet-type phosphofructokinase, aldolase, lactate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, triosephosphate isomerase, pyruvate kinase) and lysosomal proteases (cathepsins B, D, Z) in melanoma relative to normal skin. Both 2DG and LLOMe reduced the viability of A375 cells, with a markedly enhanced effect when combined, indicating a synergistic interaction as demonstrated by α-index >1. This cytotoxicity involved caspase activation, phosphatidylserine exposure, and DNA fragmentation. Caspase inhibitors, but not inhibitors of necroptosis, ferroptosis, or autophagy, reduced 2DG+LLOMe-induced cell death.

Conclusions: By demonstrating elevated expression of glycolytic enzymes and cathepsins during melanoma progression, along with the synergistic pro-apoptotic effect of the glycolytic inhibitor 2DG and lysosomal destabilizing agent LLOMe in melanoma cells, our study supports further investigation of dual targeting of glycolysis and lysosomal stability as a therapeutic strategy in melanoma.

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Synergistic antimelanoma effect of shikonin-mediated glycolysis inhibition and chloroquine-induced lysosomal destabilization in A375 melanoma cells

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Keywords: chloroquine, glycolysis, lysosomes, melanoma, shikonin

Background: Malignant melanoma is the most aggressive form of skin cancer, for which a fully effective therapy is still lacking. Melanoma cells upregulate glycolysis to sustain rapid growth and proliferation, while increased lysosomal volume and elevated activity of lysosomal enzymes cathepsins contribute to their invasiveness and chemoresistance. However, lysosomal membrane permeabilization (LMP), followed by cathepsin release into the cytoplasm, can trigger cell death. Shikonin (SH) is a natural inhibitor of the glycolytic enzyme pyruvate kinase M2 (PKM2). Chloroquine (CQ), an antimalarial drug, can induce LMP and promote the release of cathepsins B and D into cytoplasm. The aim of this study was to evaluate the antimelanoma effects of combined glycolysis inhibition by SH and lysosomal destabilization by CQ in A375 melanoma cells.

Material and Methods: The expression levels of PKM2, cathepsin B (CTSB), and cathepsin D (CTSD) genes were analyzed in patient-derived normal skin, benign nevi, and malignant melanoma samples from the publicly available GEO dataset GSE3189 using the Mann-Whitney U test in GraphPad Prism. Cell viability was assessed by mitochondrial dehydrogenase reduction assay and crystal violet staining. The type of cytotoxic interaction between the two treatments was determined using the interaction coefficient (α), calculated as α = SFsh × SFCQ/SFsh+cq, where SF represents the survival fraction of the indicated treatment. Apoptosis and necrosis were evaluated by flow cytometry following

Annexin V-FITC and propidium iodide staining.
Results: Expression of PKM2, CTSB, and CTSD was significantly higher in malignant melanoma than in normal skin or benign nevi. Both SH and CQ reduced A375 cell viability in a dose-dependent manner, with their combination exhibiting enhanced cytotoxicity. The calculated α value was greater than 1, indicating a synergistic interaction between SH and CQ. Annexin V/PI flow cytometry revealed that both agents induced apoptosis, which was significantly amplified by the combined treatment.
Conclusions: Dual targeting of melanoma-upregulated PKM2 with SH and cathepsins B/C with CQ resulted in synergistic proapoptotic effects in A375 melanoma cells, suggesting that this combined approach may represent an effective therapeutic strategy for melanoma.

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Mechanistic insights into synergistic antimelanoma activity of glycolysis inhibition and lysosomal destabilization

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Keywords: melanoma, energy metabolism, glycolysis, lysosomes, cathepsins

Background: Melanoma cells exhibit high glycolytic activity and increased lysosomal fragility. Cathepsins, overexpressed in melanoma, support tumor progression but trigger cell death upon lysosomal membrane permeabilization (LMP). This study investigated the mechanisms by which the glycolytic inhibitor 2-deoxy-D-glucose (2DG) synergized with LMP inducer L-leucyl-L-leucine methyl ester (LLOMe), to induce death of human A375 melanoma cells.
Materials and Methods: Cell viability was measured by the crystal violet test. Lysosomal integrity was evaluated using acidophilic dyes LysoTracker and acridine orange via fluorescent microscopy and flow cytometry. Fluorometric assays were used to measure mitochondrial membrane potential (JC-1), mitochondrial superoxide production (MitoSOX™ Red), glycolytic activity (pH-Xtra™ Glycolysis Assay), oxidative phosphorylation (MitoXpress® Xtra Oxygen Consumption Assay), and intracellular ATP levels (ATP assay kit). Cells were pretreated with cysteine cathepsin inhibitors (E64d, MG132), aspartic cathepsin inhibitor (Pepstatin A), lysosomal acidification inhibitor (bafilomycin A1), antioxidant NAC, and energy booster L-carnitine to further dissect the contribution of specific pathways.
Results: LLOMe synergized with 2DG to induce cytotoxicity in A375 cells. LLOMe, with or without 2DG, induced LMP, evidenced by reduced LysoTracker and acridine orange signals. Bafilomycin A1 rescued cell viability, likely by preventing LLOMe accumulation and activation within lysosomes. E64d and MG132, but not Pepstatin A, partially restored viability, implicating cysteine cathepsins in the observed toxicity. LLOMe, with or without 2DG, triggered mitochondrial depolarization and mitochondrial superoxide production, which was prevented by bafilomycin A1 and MG132. NAC rescued cells from 2DG+LLOMe-induced cytotoxicity. Metabolic assays showed that 2DG reduced glycolysis and LLOMe suppressed oxidative phosphorylation, leading to synergistic ATP depletion, while L-carnitine protected cells from the combined treatment.
Conclusions: LLOMe-induced LMP and cysteine cathepsin release lead to mitochondrial dysfunction and OXPHOS inhibition, which, together with 2DG-mediated glycolysis blockade, result in energy collapse and cell death, highlighting the therapeutic potential of dual glycolytic–lysosomal targeting.

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The ultrastructural alteration of A549 (KRASmut) and Mcf7 (KRASwt) cell lines in response to aroylacrylic acid phenylamides treatment

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Keywords: KRAS, aroylacrylic acid phenylamides, reactive oxygen species, mitochondria, lipid droplets

Background: KRAS mutation is the most frequent oncogenic alteration in human cancer, and it contributes to tumor survival and resistance to therapy. Malignant cells with a KRAS mutation cope with increased oxidative stress and maintain homeostasis by antioxidant defense mechanisms.
Material and Methods: This study was based on uncovering the cellular responses of A549 (KRASmut) and Mcf7 (KRASwt) cell lines, after 24 hours treatment with aroylacrylic acid phenylamides. The generation of reactive oxygen species (ROS) is the primary mechanism by which aroylacrylic acid phenylamides induce cell death. Comparison of the use of Annexin V and Propidium Iodide (Annexin V/PI) staining and ultrastructural analysis were performed.
Results: Significant differences were observed between treated cell lines. Initially, A549 cells showed greater resistance compared to Mcf7 cells, but despite this A549 cells suffered significant cell death after 24 hours. The heterogeneity of the cellular response between A549 and Mcf7 cells was not surprising due to the difference in their KRAS status. Transmission electron microscopy (TEM) analysis revealed ultrastructural changes, including mitochondrial damage, autophagy, and lipid accumulation in treated cells. The degree of mitochondrial damage was categorized into four grades based on the size and integrity of cristae. Autophagy was observed in both cell lines, often targeting mitochondria that were only mildly damaged. Increased lipogenesis and lipid accumulation were predominantly observed in A549 cells, indicating their metabolic adaptability. In contrast, MCF7 cells showed lipid accumulation after treatment primarily in necrotic cells.
Conclusions: Although the ability of A549 cells to induce lipogenesis and autophagy as protective strategies was ultimately insufficient, as demonstrated by the high rate of apoptosis after 24 hours. These findings suggest that KRASmut cells generate transient resistance through antioxidant defenses and lipid accumulation, delaying but not preventing cell death. Furthermore, these results indicate different metabolic vulnerabilities between KRASmut and KRASwt cells, highlighting the role of oxidative phosphorylation as a potential therapeutic target.

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Antimelanoma effect of mefloquine-mediated lysosomal destabilization and glucose deprivation in A375 melanoma cells

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Keywords: apoptosis, lysosome, mefloquine, melanoma

Background: Tumor cells often exhibit enlarged lysosomes and a high reliance on glycolysis for energy production, distinguishing them from normal cells. These features support their rapid proliferation and survival under stress. Enlarged lysosomes are more prone to membrane destabilization, while glycolytic dependence renders tumor cells vulnerable to metabolic disruption. Targeting these distinct traits with lysosome-disrupting agents and glucose deprivation offers a promising therapeutic strategy. To test this theory, we exposed A375 melanoma cell line and

normal human dermal fibroblasts (NHDF) to mefloquine, a lysosomotropic disruptive agent, in glucose-free medium.
Material and Methods: Cell viability upon treatments was determined by crystal violet and MTT tests, and meflouqine concentrations with the most pronounced anti-melanoma effects in combined treatment were selected for further investigation. The type of cell death (apoptosis, necrosis) was determined using Annexin V/PI flow cytometry and changes in lysosomal acidification were determined by flow cytometry and fluorescent microscopy after staining with Lysotracker red.
Results: Mefloquine reduced A375 cell viability in a dose-dependent manner and glucose depletion led to proliferation arrest, with their combination demonstrating higher cytotoxicity. The effect was absent in treated NHDF cells. Apoptosis was induced by both agents, as demonstrated by Annexin V/PI flow cytometry, and was significantly intensified when the agents were applied in combination. Furthermore, Lysotracker red staining revealed that lysosomal acidification decreased following the combined treatment, in contrast to the increase observed with each treatment individually.
Conclusions: Combined treatment with mefloquine and glucose deprivation elicited a potent proapoptotic response in A375 melanoma cells, while sparing NHDF cells, indicating that this dual-targeting approach may serve as a promising therapeutic strategy for melanoma.

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Targeting metabolic vulnerabilities in multiple myeloma: The role of NAD+ depletion and bone marrow adipose tissue

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Keywords: bone marrow adipose tissue, multiple myeloma, NAD+, NAMPT

Background: Multiple myeloma (MM) is a hematological cancer that develops in the bone marrow, where interactions with bone marrow adipose tissue (BMAT) support tumor growth and therapy resistance. Nicotinamide phosphoribosyltransferase (NAMPT or visfatin), an enzyme produced by both adipocytes and MM cells, is essential for NAD+ synthesis, which MM cells need for energy and survival. Thus, targeting NAMPT and NAD+ metabolism represents a perspective strategy in precision oncology, exploiting cancer cells' unique metabolic needs to enhance treatment effectiveness. This study investigates the effects of NAMPT inhibition on MM cell metabolism within the bone marrow microenvironment.
Materials and Methods: Bone marrow samples were obtained from non-cancer (n=11) and MM patients (n=10) and used for investigation of BMAT and plasma cells in downstream assays in hypoxic (3% O2) and normoxic (21% O2) conditions. The FK866 (NAMPT inhibitor) was added to cultures of myeloma cell lines (AMO-1, OPM2 and U266) in normoxia and hypoxia.
Results: The results indicate that the BM of patients with MM has a significantly lower proportion of BMAT than that of non-cancer BM, while the distribution of CD138+ plasma cells is increased in cells isolated from the BMAT compartment. In vitro co-culture assays of bone marrow adipocytes and MM cells show that pre-treatment of MM cells with FK866 increase their de-lipidation capacity. FK866 at a concentration of 1 nM leads to a significant decrease in the number of MM cells in hypoxia, but not in normoxia. Moreover, the presence of FK866 increases the clonogenic potential of myeloma cells in hypoxia, while in normoxia, a decrease in clonogenic potential was determined. Cell cycle analysis shows that FK866 under hypoxia decreases actively dividing cells (G2-M) and increases cells in the resting phase (G0). Under normoxia, FK866 reduces cells in the DNA synthesis and mitosis phases (S-M) but increases cells in

G0 and G2 phases. FK866 also promotes mitochondrial biogenesis in hypoxia and stimulates the expression of genes related to NADH dehydrogenase, HIF-1α, and Nanog, especially at 0.5 nM concentration.
Conclusions: Collectively, these results show that the FK866 inhibitor influences the cell cycle, mitochondrial biogenesis, and crosstalk with BMAT in multiple myeloma cells, with its effects varying based on oxygen availability. This underscores FK866's potential to regulate NAD+ metabolism and positions it as a therapeutic agent targeting metabolic pathways in MM.

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SESSION 14

INNOVATIVE MODELS AND APPROACHES IN CANCER RESEARCH

P91

Trophoblast-derived extracellular vesicles enhance cisplatin-induced apoptosis in ovarian cancer A2780 cells via Bax/Bcl-2 modulation

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Keywords: Ovarian cancer, extracellular vesicles, cisplatin, apoptosis, trophoblast

Background: Ovarian cancer is the leading cause of death among gynecological malignancies, associated with frequent chemoresistance. Extracellular vesicles (EVs) from the placenta have recently shown therapeutic potential by altering the ovarian tumor cell phenotype, though the precise mechanisms remain unclear. The aim of this study was to investigate whether EVs from trophoblast cells (TC-EVs) could affect the viability of ovarian cancer A2780 cells and improve their sensitivity to cisplatin.
Material and methods: TC-EVs were isolated from the conditioned media of the trophoblast cell line HTR-8/SVneo using differential ultracentrifugation. Analysis of the concentration and size distribution of total, as well as CD9- and CD81-positive TC-EVs, was performed using a nanoparticle tracking analyzer. Ovarian cancer cells A2780 were preconditioned with TC-EVs (50 µg/mL protein) for 24 or 48 hours, followed by treatment with 15 µM cisplatin. Cell viability was assessed by MTT assay, and changes in the expression of pro-apoptotic Bax and anti-apoptotic Bcl-2 mRNA were quantified using qPCR.
Results: At the 24h treatment, cisplatin treatment markedly reduced the viability of A2780 cells, and there was no difference between TC-EVs preconditioned cells and cells exposed to cisplatin alone. However, at the 48-hour treatment, preconditioning with TC-EVs produced a statistically significant additional decrease in cell viability compared to cisplatin alone, indicating a potential sensitizing effect of TC-EVs. This was accompanied by increased Bax and decreased Bcl-2 expression, resulting in a higher Bax/Bcl-2 ratio in TC-EVs preconditioned cells, indicating a pro-apoptotic shift.
Conclusions: Trophoblast-derived EVs display in vitro cisplatin-sensitizing effects in ovarian cancer cells in a time-dependent manner, likely by modulating pro-apoptotic pathways. This suggests that TC-EVs might play a role in improving the effectiveness of platinum-based ovarian cancer chemotherapy.

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Assessment of the Efficacy of a Biomedical Cell Product Production Technology Using Tumor-Infiltrating Lymphocytes Derived from Surgical Specimens of Patients with Advanced Melanoma

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Keywords: adoptive cell therapy, melanoma, tumor-infiltrating lymphocytes

Background: Adoptive cell therapy of cancer using tumor-infiltrating lymphocytes (TIL) has demonstrated significant potential, even against refractory tumors. For instance, in melanoma, the rate of objective responses ranges from 40% to 70%, and approximately 10–15% of patients with refractory melanoma achieve durable complete remission. The aim of this study was to evaluate the efficacy of our developed domestic laboratory technology for producing a biomedical cell product based on TIL (a TIL-based biomedical cell product, or BMCP-TIL), intended for treating patients with advanced melanoma. **Material and Methods:** Operative tissue samples were obtained from 12 patients. Under conditions conducive to eliminating tumor cells, at least 10^5 viable TILs were isolated from each sample. During activation, gamma-irradiated peripheral blood cells from healthy donors, irradiated with a dose of 50 Gy, were used as feeder cells, followed by the addition of anti-CD3 antibody (OKT3) and interleukin-2. Rapid amplification of TILs was performed in standard culture flasks until clinically relevant numbers—equal to or exceeding 10^9 viable TILs—were achieved. Subsequently, gamma-interferon (IFNγ) production levels were assessed. Following this, TILs were purified from culture medium components and transferred into infusion bags containing Ringer's solution to produce the BMCP-TIL. Finally, the resulting TILs were counted and analyzed for viability and cluster of differentiation (CD) surface marker expression. **Results**: A complete cycle of BMCP-TIL production was successfully carried out for operative material from 10 out of 12 patients with advanced melanoma, with the process typically taking 7–8 weeks. Amplified TILs exhibited IFNγ production activity ranging from 50 to 250 pg/mL. The number of TILs in the final BMCP-TIL product ranged from (1–23)×10^9 cells, with viability exceeding 98%. Phenotypic analysis revealed the following distribution of differentiation clusters: CD3+– (85–99%), among which CD8+ – (26–95%) and CD4+ – (1–67%), while regulatory T cells (CD25+FoxP3+) accounted for no more than 0.2%. **Conclusions:** The high efficiency of the first Russian laboratory technology for producing BMCP-TIL for treating patients with advanced melanoma has been demonstrated.

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Thyroid-cell derived EVs harbour surface thyrotropin-receptor and intravesicular thyroglobulin offering a novel approach for detecting thyroid cancer recurrence

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Keywords: Cell line, Extracellular vesicles, Tetraspanin, Thyroid cancer, Thyroglobulin, Thyrotropin receptor

Background: Thyroid cancer (TC) poses diagnostic challenges in differentiating benign from malignant tumors and detecting recurrence, which occurs in 21–27% of patients. Analysis of serum thyroglobulin (Tg) can detect recurrence early, but the marker is not applicable in patients harboring Tg antibodies (TgAt) due to TgAb interference in standard Tg

detection tests. Extracellular vesicles (EVs) present a promising target for non-invasive diagnostics as they are enriched in transmembrane proteins such as tetraspanins and tissue-specific markers. We investigated whether thyroid-cell derived EVs retain tissue specific surface characteristics (thyrotropin receptor – THS-R) and intravesicular cargo (Tg) that can enable their use in recurrent TC detection.
Materials and Methods: EVs were extracted via differential ultracentrifugation (dUC) from FBS-free cell culture media of normal thyroid (Nthy-Ori 3-1) and cancer cell lines (TPC-1 and OCUT2) and patient plasma (benign, malignant, recurrent). EVs number, diameter and zeta potential were assessed using Nanotracking Analysis (NTA), and their presence was confirmed by Transmssion electron microscopy (TEM). Tetraspanin CD63 – an EV marker, TSH-R, and Tg – thyroid specific protein markers were detected via Dot Blot and Western Blot.
Results: NTA detected 130–160 nm particles, in all samples implying that EV pellets were enriched with small EVs with TEM analysis confirming that conclusion. Zeta potential differed between EVs from Nthy-Ori 3-1 cell line and TPC-1-devoid EVs. CD63 was present in EVs from all cell lines and patient plasma. The expression of TSH-R was higher in Nthy-Ori 3-1 cell lysates, compared to TPC-1 and OCUT2, corresponding to their differentiation state. Consistent with this, TPC-1 EVs were positive while OCUT2 EVs were negative of TSH-R, however Nthy-Ori 3-1 EVs were deprived of TSH-R. Tg was confirmed in cell lysates and EVs from Nthy-Ori 3-1 and TPC-1 EVs but absent in OCUT2 cells and EVs. EVs from plasma of patients with thyroid tumors as well as recurrence were positive for both TSH-R and Tg. The highest Tg signal was observed in a malignant sample with larger tumor size.
Conclusions: This pilot study highlights the potential of using CD63 and TSH-R proteins for isolating thyroid-specific EVs. The presence of thyroid-specific TSH-R and intravesicular Tg in all patient samples paves the way for studies of larger sample cohorts to address early recurrence detection.

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3D spheroid cell models reveal the ovarian cancer secretome

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Keywords: cytokines, hedgehog signaling pathway, ovarian neoplasms, spheroids

Background: Ovarian cancer is among the most common and lethal gynecological cancers. Three-dimensional (3D) cell cultures provide a physiologically relevant model to study tumor biology and test therapies. The Hedgehog (HH) signaling pathway, often aberrantly activated in cancers including ovarian cancer, plays a critical role in tumor progression and chemoresistance. In this study, 3D spheroids were established using ovarian cancer cell lines (OVCAR8, OVCAR3, OVSAHO, IGROV1, SKOV3) and an ovarian fibroblast line (HOF). Spheroid formation was monitored over five days, and viability was assessed with a three-color live/dead assay. OVCAR8, a high-grade serous adenocarcinoma line, was selected for further investigation. To explore HH signaling, OVCAR8 cells were transfected with GLI2 and cultured as spheroids. A protein array of 108 secreted cytokines and chemokines revealed four potential GLI2 transcriptional targets.
Materials and Methods: Ovarian tumor cell lines and the ovarian fibroblast line were cultured in suspension using U-bottom well plates to promote spheroid formation over five days. Viability was assessed by staining spheroids with a live/dead detection kit, and quantifying cell death via red fluorescence. Supernatants were collected and analyzed by protein array to profile secreted factors.
Results: Based on superior spheroid morphology and higher viability, OVCAR8 and OVSAHO were chosen for further study. Compared to OVSAHO, OVCAR8 formed larger, more compact, and uniformly shaped spheroids. At a seeding density of 5,000 cells, OVCAR8 spheroids showed significantly higher viability by live/dead assay. Protein array analysis of conditioned media from GLI2-overexpressing OVCAR8 spheroids identified DKK-1, EMMPRIN, IL-6, and IL-8 as potential GLI2 transcriptional targets.
Conclusions: All ovarian cancer cell lines successfully formed 3D spheroids, with OVCAR8 demonstrating the highest viability. Morphological and viability assessments indicated 5,000 cells as the optimal seeding density for spheroid formation. Compared to OVSAHO, OVCAR8 spheroids were larger, more compact, and more uniformly shaped, with significantly higher viability. Protein array analysis of GLI2-transfected OVCAR8 spheroids identified DKK-1, EMMPRIN,

IL-6, and IL-8 as candidate GLI2 targets. Future qPCR validation will confirm the transcriptional regulation of these genes by GLI2.

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Bridging the in vitro–in vivo gap: Tumor engineering for relevant and reliable cancer research

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Keywords: carcinoma, osteosarcoma, doxorubicin, cisplatin

Background: One of the major challenges in the development of novel, effective, and safe anti-tumor drugs is the translation of results from in vitro studies to in vivo models, and subsequently to clinical trials. A promising approach to address this in vitro–in vivo gap is tumor engineering. Accordingly, the aim of this work was to develop three-dimensional (3D) tumor models using biomaterials as cell carriers to mimic extracellular matrices (ECM) followed by cultivation in a perfusion bioreactor, that imitates the environment of highly vascularized tissues.
Material and Methods: Alginate microfibers and microbeads with immobilized human carcinoma cells (SiHa, HeLa, NCI-H460 and MCF-7) were obtained by extrusion techniques, while macroporous composite carriers for osteosarcoma cells (murine K7M2-wt) based on alginate and bioactive inorganic particles (e.g. hydroxyapatite) were prepared by gelation and freeze-drying followed by manual cell seeding onto the carriers. Cells in carriers were cultivated in perfusion bioreactors under continuous medium flow for up to 10 days. To evaluate these 3D models for drug screening, microfibers with cells were treated with cisplatin or doxorubicin (0.5-50 μM), while cell-seeded macroporous carriers in bioreactor cultures were treated with doxorubicin (1.84 μM) starting on day 7 and continued for 3 consecutive days. The cell metabolic activity was assessed by the MTT assay, morphology by scanning electron microscopy and histology, and ECM by reticulin staining.
Results and Conclusions: Cells were successfully immobilized in microbeads (diameter: ~300 μm) and microfibers (diameter: 300-500 μm), while the macroporous carriers provided cell adherence (seeding efficiency: above 80%). After bioreactor cultivation the cells stayed viable, spontaneously formed spheroid-like structures, and exhibited higher metabolic activity than static controls. Evaluation of these models for rapid drug screening showed that the immobilized cells in alginate hydrogels exhibited higher resistance than the cells in 2D cultures. Furthermore, assessment of drug effects on cells within spheroid-like structures treated in a clinically relevant schedule showed negligible effects compared to the untreated 3D cultures implying a resemblance to the in vivo drug resistance. Overall, the results of these studies demonstrated the potential of developed 3D models based on biomimetic perfusion bioreactors and alginate cell carriers for cancer research and anticancer drug screening.

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Modelling pancreatic carcinoma *in vitro*- critical morphological and functional differences between 2D and 3D mono- and co-cultures

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Keywords: disease modelling, in vitro cultures, pancreatic carcinoma

Background: Pancreatic ductal adenocarcinoma (PDAC) is a major histological subtype of pancreatic cancer with high proliferative and metastatic potential and a low survival rate with a 5-year relative survival of 12%. Although histologically classified as adenocarcinoma, like breast, colorectal, and prostate cancers, commonly used treatments that are effective against these carcinomas are not effective against PDAC. There are many reasons for this including molecular mechanisms linked to drug resistance, the presence of cancer stem cells and the role of the tumor microenvironment (TME).
Material and Methods: Since PDAC cells actively interact with components of their TME it is reasonable to perform in vitro investigation using more complex models by including some components of the TME. We have employed two different PDAC cell lines together with fibroblast in contact and contact-free 2D culture conditions and 3D PDAC spheroids with or without fibroblast and performed a comparative analysis of basic cellular processes.
Results: Fibroblasts had a moderate or no inhibitory effect on PDAC cell viability in both contact and non-contact 2D co-cultures, but significantly reduced colony formation in both cell lines tested and strongly affected sphere size in 3D culture as reflected in significantly smaller and more compact sphere diameter. Both wound scratch and Transwell assay showed that the efficiency of migration is significantly different if studied in co-cultures, or when PDAC cells are grown in fibroblast conditioned medium. Pancreatic carcinoma cells more likely migrate together as a group filling the wounded area, whereas fibroblast have high migratory potential and dominantly migrate as single cells occupying the gap, however it is very difficult to determine the exact role of each cell type in the co-culture model. Similarly, pancreatic cells significantly stimulate migration of fibroblast and influence collagen I production. Finally, in 3D spheroid co-culture cells were organized similarly to in vivo conditions where PDAC cells were in the centre of the sphere surrounded by highly proliferative Ki67 positive fibroblasts.
Conclusions: There are significant differences observed between basic cellular processes of PDACs when cultured in mono- or co-cultures with activated fibroblast and these differences are even more obvious when comparing 2D and 3D conditions. This should be taken into consideration when using in vitro models for studying disease mechanisms and drug testing.

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Development of bioactive environment for 3D osteosarcoma *in vitro* cultures

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Keywords: alginates, porosity, tissue scaffolds

Background: Osteosarcoma (OS) is a primary bone cancer that affects mostly children. There have been minimal advancements in therapeutic strategies over the past four decades due to the weaknesses of standard models used in OS research, namely two-dimensional (2D) cell cultures and animal models. In recent years, three-dimensional (3D) cell culture models have gained significant attention as promising platforms for cancer research. The aim of this study was to develop macroporous composite alginate cell carriers with embedded bioactive glass (BG) particles as a bioactive environment that could stimulate osteosarcoma cells to express their typical morphology and metabolic

functions as observed in vivo.
Materials and methods: Two types of BG particles, SBA2 (48SiO2-18Na2O-30CaO-3P2O5-0.43B2O3-0.57Al2O3 mol%) and 47.5B (47.5SiO2-10Na2O-10K2O-10MgO-20CaO-2.5P2O5 mol.%) were assessed regarding cytocompatibility with murine OS K7M2-wt cell line by MTT assay, as well as bioactivity to transform into hydroxyapatite (HA) in cell culture medium by scanning electron microscopy (SEM) and energy dispersive X-ray (EDS) analysis. Then, the two types of BGs were used to produce and optimize composite cell carriers obtained by gelation of mixtures composed of 2 wt.% Na-alginate, 2 wt.% BAG and 0.045 – 0.075 wt.% CaCl2, followed by freezing, freeze-drying and rehydration. Then, the scaffolds were seeded with the OS cells (K7M2-wt, 15×106 cells cm-3 scaffold volume) and cultivated for 7 days under static conditions. The cell carriers were examined regarding porosity, BG distribution by SEM, cell metabolic activity by MTT assay and morphology by SEM and histological analysis.
Results and conclusions: Bioactivity of the 47.5B and SBA2 glass powders was detected on day 2 and 5, respectively, while the MTT assay showed biocompatibility of both BGs. The best cell carriers were obtained using mixtures with the composition: 2 wt.% Na-alginate, 2 wt.% BG (SBA or 47.5B) and 0.060 wt.% CaCl2. The cells were successfully seeded on both cell carriers, but higher cell seeding efficiency was observed on alginate/47.5B carriers (92%) compared to alginate/SBA scaffolds (72%). After short term cultivation, the cells stayed viable and spontaneously formed aggregates in the presence of both BG particle types. The obtained results show that alginate-based cell carriers with BG particles support OS cell culture with alginate/47.5B offering higher cell-seeding efficiency and bioactivity.

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Engineered 3D *in vitro* model based on Ba-alginate microfibers for rapid anticancer drug screening

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Keywords: cells, immobilized, cisplatin, drug resistance, drug screening assays, antitumor; etoposide, vinorelbine

Background: Reliable preclinical models in cancer research are crucial for improving anticancer drug development. Existing models, such as two-dimensional (2D) cell cultures and animal studies, often fall short in accurately replicating human cancer biology, leading to significant differences in drug efficacy between preclinical studies and clinical trials. In this work, a simplified 3D cancer cell culture model in the form of Ba-alginate microfibers with immobilized cancer cells was developed, optimized and validated for anticancer drug screening.
Material and Methods: Alginate microfibers with immobilized human non-small cell lung carcinoma cells (NCI-460 cell line, ATCC® HTB-177™) were obtained by manual extrusion of cell-alginate suspension (4×106 cells cm-3, 2 wt. % Na-alginate solution) through a 25-gauge needle into the gelling solution containing 45 mM Ba2+ (BaCl2·2H2O). After 1 min of gelation, the obtained microfibers were washed with culture medium RPMI-1640 and cultured up to 7 days. Cells in microfibers, as well as cells in 2D cultures, were treated with different concentrations of cisplatin (0.5–50 μM), etoposide (0.5–500 μM), or vinorelbine (1–1000 μM) either 24 or 72 h after immobilization, while untreated cells served as the control. Microfibers were evaluated for cell viability by live/dead staining and metabolic activity by MTT assay, while the expression of drug resistance–related genes was assessed by using quantitative real-time PCR.
Results and Conclusions: The cells remained viable and metabolically active, forming cell aggregates during the 7-day cultivation period. Analysis of cell sensitivity to anticancer drugs revealed increased drug resistance in 3D cultures compared to 2D cultures across all treatment groups (cisplatin, etoposide and vinorelbine). Additionally, the gene expression analysis showed a significant upregulation of drug resistance–related genes in 3D cultures, including a 3.2-fold increase in MDR1 and a 2.7-fold increase in ABCG2 expression following cisplatin treatment, and a 1.5-fold increase in ABCC1 expression after vinorelbine exposure, whereas these changes were not observed in 2D cultures. Overall, the obtained results demonstrate the potential of the proposed 3D cancer model for rapid, relevant and high-throughput anticancer drug screening.

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The establishment of an apoptosis-induced compensatory proliferation model: challenges, optimization, and variabilities between different mouse cancer models

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Keywords: animal experimentation, apoptosis, cell proliferation, colorectal neoplasms, melanoma, organoids

Background: Although apoptosis of cancer cells is typically considered a desirable therapeutic effect, numerous studies have emerged describing the paradoxical relationship between chemotherapy-induced apoptosis and an increased cell proliferation rate in the tumor microenvironment. Notably, apoptosis triggers the activation of protective mechanisms that tend to compensate for cell loss in tumor tissue, unveiling the phenomenon described as compensatory proliferation. To better understand the strategy of cell death-induced proliferation in tumors, we developed a suitable advanced mouse model that appropriately mimics the communication between dying and living cells within the tumor mass. We have also established mouse-derived organoids from isolated tumors and used transcriptional analysis to assess the gene expression similarity and evaluate the potential of the formed organoids as 3D models to replace animal studies in the future.
Material and Methods: The compensatory proliferation model was developed by subcutaneous inoculation of a mixture of live and dead cancer cells of different origins into mice (MC38 or B16 cells into C57BL/6 mice; CT26 cells into BALB/c mice). The dead cells were collected after in vitro treatment with 5-FU (MC38 and CT26 cells) or paclitaxel (B16 cells). The number of live cells and the live:dead cells ratio in the mixture were varied. Both tumors and organoids from MC38 tumor model were further analysed by RNA-seq.
Results: While the optimization of MC38 compensatory proliferation model in C57BL/6 mice was successful, and live:dead cells mixture led to the earlier tumor onset, faster growth, and higher incidence rate compared to the group that received only live cells, CT26 model in BALB/c mice failed due to low incidence rate in all groups. Conversely, the mixture of live and dead B16 cells induced smaller tumors compared to the group that received only live cells, indicating a strong immune response of animals to dead melanoma cells. The transcriptional analysis of isolated tumors and their matching organoids from the MC38 compensatory proliferation model showed over 80% homology in gene expression.
Conclusions: The obtained data revealed that the establishment of a compensatory proliferation model depends on multiple factors, such as the origin, growth rate, invasiveness, and immunogenicity of the cell lines used.

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3D Bone-Mimetic Cell Carriers for Assessing the Effects of 5-Fluorouracil and Doxorubicin on SAOS2 Cells

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Keywords: 3D bone-mimetic cell carriers, SAOS2, 5-FU, doxorubicin, human osteosarcoma

Background: The SAOS2 cell line is a human osteosarcoma cell line, widely used as a model system for studying bone cancer due to its osteoblastic phenotype and capacity to mineralize the extracellular matrix under specific conditions. The development of physiologically relevant in vitro models is crucial for predictive drug screening for osteosarcoma, given the persistent challenges of chemoresistance and recurrence. Therefore, the aim of this study was to assess the effects of two chemotherapeutics, 5-fluorouracil (5-FU) and doxorubicin, on SAOS2 cells in a 3D model based on macroporous composite cell carriers (alginate and hydroxyapatite) that mimic the bone microenvironment.
Materials and Methods: Two different approaches for drug testing on cells were conducted. In the first approach, cells were manually seeded onto carriers and treated 24 hours post-seeding with either 5-FU or doxorubicin for an additional 24 hours. In the second approach, SAOS2 cells were allowed to self-organize on the carrier over 7 days prior to 24 hours long treatments. Cell viability was evaluated twice in both approaches: after the treatment was finished and following a 7-day recovery period without any applied treatments.
Results: The cells were successfully seeded on carriers as individual cells. In the first approach, 5-FU reduced viability to 60% post-treatment, with full recovery (100%) observed after 7 days. In contrast, doxorubicin decreased viability to 85% initially, with a further reduction to 50% after a 7-day recovery period. In the second approach 5-FU had no significant effect on cell viability compared to the untreated 3D culture which served as a control in both time points (24 hours and 7 days), indicating resistance in the self-assembled 3D culture. In contrast, doxorubicin reduced viability by approximately 50%, which is significantly lower than the viability observed in individual cells in the first approach. Evaluation of long-term recovery revealed that 5-FU did not affect cell viability, whereas the doxorubicin-induced reduction persisted, though to a lesser extent than after treatment at day 7.
Conclusions: These findings highlight the impact of 3D culture cell aggregation on drug sensitivity and underscore the relevance of cell carrier-based models for future osteosarcoma drug testing.

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Localized rose bengal delivery via electrospun poly(lactic-co-glycolic acid) mats for post-surgical melanoma therapy

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Keywords: skin cancer, residual tumor, rose bengal, nanofibers, wound dressing

Background: Cutaneous malignant melanoma is the deadliest form of skin cancer, with postoperative recurrence rates ranging from 10–70%, depending on the stage. Recently, rose bengal (RB), a xanthenic dye with anticancer and immunogenic properties, has shown promise in intralesional melanoma therapy. PV-10, a 10% RB solution in saline, is undergoing phase 3 trials, highlighting its therapeutic potential. However, the persistence of residual tumor cells remains a challenge, requiring sustained RB delivery after surgery. The aim of this study was to develop a wound dressing based on poly(lactic-co-glycolic acid) (PLGA 50:50) mats loaded with RB for localized and prolonged release.
Materials and Methods: The cytotoxic effect of RB was evaluated by determining the half-maximal inhibitory concentration (IC_{50}) on human cell lines: keratinocytes (HaCaT), melanoma (A375), and fibroblasts (MRC5). Cells were exposed to different concentrations of RB in PBS (20 to 400 µM) for 24 and 48 h in monolayer culture, and cellular metabolic activity was assessed by the MTT assay. PLGA mats (40 wt% in DCM/DMF 1:1) loaded with 5 wt.% RB were produced using the blend electrospinning method under the flowrate 0.5 ml/h, distance from the collector set at 15 cm, and voltage of 18 kV. The mats (2 cm diameter) were additionally functionalized via overnight incubation in RB solutions (200 or 400 µM). The functionalized mats were analyzed by scanning electron microscopy and Fourier-transform infrared spectroscopy (FTIR), while the RB release concentration was evaluated over 48 h in PBS by UV-Vis spectroscopy at the wavelength 549 nm.

Results and Conclusions: A375 and MRC-5 cells were sensitive to RB (IC_{50} ~200 µM), while HaCaT cells did not exhibit significant sensitivity. Electrospun PLGA/RB mats (nanofiber diameter: 300 nm) were successfully fabricated, although notable dimensional shrinkage (~72%) of the mats was induced after functionalization (based on IC_{50} values). The release study demonstrated a higher initial release of RB from mats functionalized with 400 µM during the first 24 h, whereas the final concentration released after 48 h was comparable between both materials (~20 µM), showing no significant difference. These findings support the use of electrospun PLGA mats as a feasible strategy for RB delivery, offering controlled and prolonged release upon application followed by sustained local diffusion for targeted post-surgical melanoma therapy.

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Enhancing the Zebrafish Xenograft Methodology at the Institute of Molecular Genetics and Genetic Engineering (IMGGE)

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Keywords: lung cancer, patient-derived xenografts, response to therapy, xenograft, zebrafish

Background: Lung cancer remains the leading cause of cancer-related mortality worldwide, largely due to tumor heterogeneity and the development of drug resistance, which limit treatment success. To address these challenges, rapid, reproducible, and cost-effective preclinical models are essential for advancing translational research. Among these, the zebrafish (Danio rerio) xenograft model offers unique advantages. Their optical transparency, rapid development, high fecundity and absence of adaptive immune system during the first three weeks of life enable real-time, in vivo monitoring of tumor cell proliferation, migration, angiogenesis, and response to therapy. Our overall aim is to introduce a patient-derived xenograft model in zebrafish to monitor therapeutic responses in lung cancer patients.
Material and Methods: We are implementing and optimizing the zebrafish xenograft methodology based on the protocol we learned at Rita Fior's laboratory, using the HCT116 colorectal cancer cell line as a proof-of-concept model. Tumor cells were expanded, fluorescently labeled, and prepared at a standardized concentration for injection. Cells were injected into the perivitelline space (PVS) of 2-day-old embryos using a fine glass needle, with injection parameters (pressure and injection time) dynamically adjusted during the procedure to ensure accurate cell delivery and minimize embryo damage. After injection, zebrafish embryos were kept at 34°C and tumor size and shape were monitored by fluorescence microscopy for 4 days.
Results: In our first attempt, 10 larvae were confirmed as successfully injected at 1 day post-injection (dpi). By 4 dpi, only 3 larvae survived. Among these, one had a distinct tumor in the PVS, which is a favorable outcome. One exhibited pronounced edema, and the third contained only dispersed tumor cells in the PVS without a clearly formed tumor. These preliminary results highlight the need for further optimization of injection parameters and larval handling to improve survival rates and ensure consistent tumor engraftment.
Conclusions: Our ongoing efforts to optimize the zebrafish xenograft methodology aim to establish a solid foundation for future translational studies at IMGGE. This platform is adaptable to other cancer cell types, including patient-derived cells from various sample types. Once the protocol is fully refined, it will allow rapid, reproducible, and scalable evaluation of tumor behavior and therapeutic responses.

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Development of a Clinically Relevant Temozolomide-Resistant Human Glioblastoma A-172 Cell Line

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Keywords: drug resistance, glioblastoma, temozolomide

Background: Glioblastoma is the most aggressive and deadly form of brain cancer, with a median survival of approximately 14 months. A major factor contributing to an unfavorable prognosis is the development of resistance to temozolomide (TMZ), the current first-line chemotherapeutic drug for glioblastoma. Thus, developing reliable preclinical models of TMZ resistance is essential for evaluating clinically relevant therapeutic strategies.
Material and Methods: To generate an in vitro resistance model that mirrors the cyclical TMZ administration used in the Stupp regimen (5 days of treatment followed by a 3-week break), we implemented an adapted treatment schedule compatible with cell culture conditions. The TMZ-sensitive human glioblastoma cell line A-172 was treated with 10 µM TMZ for two consecutive cycles of 3-day exposure, each followed by a 3-week recovery phase. Sensitivity to TMZ was evaluated in both parental cells and TMZ-exposed cells via MTT assay. The MTT assay was performed on cells treated with TMZ once, twice, and three times (i.e., single, double and triple TMZ treatment). To further characterize the TMZ-exposed cells, we assessed changes in cell migration (wound healing assay), EGFR, CD44, SOX2 and CD133 expression (immunostaining), and cellular senescence (beta-galactosidase activity assay after exposure to 10 and 50 µM TMZ during three days).
Results: Relative to the parental cells, the TMZ-exposed population showed progressively higher IC50 values, in single, double and triple TMZ treatment, suggesting a gradual development of resistance. In the wound healing assay, resistant cells showed reduced motility, closing the wound at approximately half the rate of the parental line after 72 h. Immunofluorescence assays showed a minor increase in EGFR expression and a slight reduction in CD44 expression, while SOX2 and CD133 levels remained unchanged. In the beta-galactosidase activity assay after exposure to both 10 and 50 µM TMZ, the resistant cell line showed a reduced proportion of senescent cells relative to the parental line.
Conclusions: The cyclic TMZ regimen adapted for in vitro use successfully induced resistance in A-172 cells, generating a cell line with reduced migratory capacity, slightly increased EGFR expression, decreased CD44 expression, and reduced senescence.

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A 3D in vitro osteosarcoma model based on porous scaffolds and a perfusion bioreactor for preclinical applications

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Keywords: 3D cell culture, biomimetic materials, bioreactors, bone cancer, calcium alginate, hydroxyapatite

Background: Osteosarcoma is a primary bone tumor with relatively low incidence in the human population, as 3 to 4 people per million are diagnosed with this disease each year. It is an aggressive tumor with a high tendency to metastasize to other distant organs, most commonly the lungs. The slow and inefficient process of anti-cancer drug

discovery, mainly due to the use of inadequate preclinical models (cell monolayers and animals) along with the limited understanding of osteosarcoma biology, necessitates the development of more in vivo–like cell culture models. In order to address this issue, we developed a three-dimensional (3D) in vitro osteosarcoma model based on scaffolds and a perfusion bioreactor ("3D Perfuse", Innovation Center at the Faculty of Technology and Metallurgy, Belgrade, Serbia).
Materials and methods: The scaffolds are designed to imitate the bone environment where osteosarcoma originates, both in structure and composition. Therefore, the scaffolds are porous and composed of alginate and hydroxyapatite (2 wt.% each), representing organic and mineral phases of the bone, respectively. Perfusion bioreactors enable efficient mass transport to the cells and introduce biophysical stimuli in the form of hydrodynamic shear stresses. The scaffolds were seeded with murine osteosarcoma cells (K7M2-wt,15x106 cells/cm3 of the scaffold volume) and cultivated in perfusion bioreactors at a superficial medium velocity of 40 µm/s, while static conditions served as a control.
Results: During 7 days of cultivation under both perfusion and static conditions, the cells proliferated and were metabolically active. Moreover, the cells spontaneously aggregated into compact spheroid-like structures, with an average size of 140 µm under both conditions. Still, under perfusion conditions the cell aggregates were more abundant and exhibited an elongated shape, probably due to shear stresses acting calculated to be 2 mPa in average. Additionally, the cells secreted more reticulin fibers under perfusion conditions than under static conditions. In perfusion cultures, cell aggregates were distributed across the whole thickness of the scaffold, in contrast to static cultures in which the cell aggregates remained only in the top part of the scaffolds, correlated with the limited transport of nutrients.
Conclusions: Our model supported the tumor-like behaviour of osteosarcoma cells and could be further utilized in long-term studies and drug testing.

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Short- and Long-Term Studies of a 3D Osteosarcoma Cell Culture Model for Doxorubicin Drug Screening

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Keywords: 3D in vitro models, bone cancer, osteosarcoma, doxorubicin, anticancer drug screening

Background: Osteosarcoma is an aggressive malignant bone tumor that mainly develops in children and adolescents during periods of rapid bone growth and has a high tendency to metastasize. Despite intensive treatment involving neoadjuvant multi-agent chemotherapy, surgical resection, and adjuvant multi-agent chemotherapy, the tumor recurs in over 40% of cases. One major obstacle in improving treatment outcomes is the lack of effective preclinical models that can better reflect the complex tumor environment found in patients. A promising approach is to develop 3D osteosarcoma cell culture models using bone-mimicking scaffolds combined with biomimetic perfusion bioreactors to ensure efficient mass transport and adequate biophysical signals. This study aimed to evaluate a previously developed 3D culture model for osteosarcoma cells based on macroporous composite scaffolds (2 wt.% alginate and 2 wt.% hydroxyapatite) in conjunction with a perfusion bioreactor for anticancer drug screening.
Materials and methods: Murine osteosarcoma cells (K7M2-wt) were seeded onto the scaffolds (15x106 cells cm-3 of scaffold volume) and cultivated under static conditions for one day. Three experimental studies were performed using the anticancer drug doxorubicin. In the first, following 1 day of static cultivation, the drug (1 µg cm^{-3}) was applied for 1 day under continuous flow in the "3D Perfuse" bioreactor (0.27 cm^3 min^{-1}; 40 µm s^{-1}). In the second, scaffolds were cultured for 7 days under the same conditions to allow spontaneous formation of spheroid-like structures, followed by 3-day doxorubicin treatment. Additionally, a long-term model was introduced to better reflect clinical settings. Spheroid-like structures were exposed to the drug for 3 days, followed by a 21-day recovery period under perfusion, simulating intervals between chemotherapy cycles in patients. The cultivated scaffolds in all studies were then assessed by performing histological analysis and MTT assay to measure cell metabolic activity.
Results: Osteosarcoma cells cultured in a 3D environment were less sensitive to the drug compared to those in 2D, reflecting the chemotherapy resistance observed in patients.
Conclusions: This 3D osteosarcoma cell culture model is suitable for short-term drug screening and potentially for

long-term studies.

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Characterization of osteosarcoma stem cells using quantitative live-cell imaging and 3D spheroid analysis

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Keywords: Cancer Stem Cells, Cell Migration, Osteosarcoma, Patient Samples, Three-Dimensional Cell Culture Techniques, Tumor Aggressiveness

Background: Osteosarcoma is the most common primary malignant bone tumor in children and adolescents, characterized by aggressive progression, high metastatic potential, and frequent recurrence. Cancer stem cells (CSCs) are believed to contribute to tumor initiation, therapy resistance, and disease progression due to their self-renewing and invasive properties. This study aims to characterize CSCs isolated from a single osteosarcoma patient at three different stages of disease: biopsy at diagnosis, post-chemotherapy resection, and resection after recurrence.
Material and Methods: Using the HoloMonitor live-cell imaging system, we monitored cells over 48 hours and quantitatively analyzed cell migration and morphology, assessing optical thickness, surface area, perimeter, shape irregularity, and wound width, reflecting dynamic cell behavior and phenotypic heterogeneity. Additionally, to evaluate the same samples in a 3D context, we generated spheroids from first-generation CSCs, which were isolated using cell strainers and fixed in 4% PFA in PBS. Spheroids were assessed using the W8 Physical Cytometer, a technology for biophysical characterization and physically-based sorting of sphere-like 3D cultures. This method enabled precise measurement of spheroid diameter, mass density, and weight, providing valuable insight into tumor compactness and 3D structural organization. Data were processed using the HoloMonitor App Suite for 2D imaging and Libra Software for 3D spheroid data, followed by statistical analysis.
Results: Results revealed that CSCs from the recurrent stage migrated significantly faster and exhibited increased morphological complexity, characterized by greater shape irregularity, larger perimeter size, and the thinnest optical thickness. Additionally, they formed spheroids that were more compact and homogeneous compared to those from earlier stages. Significant differences in spheroid mass density, weight, and diameter were identified between samples obtained at diagnosis and those from recurrence. Comparative analysis revealed consistent, complementary findings, supporting the hypothesis that CSCs from recurrent tumors represent a more aggressive and invasive phenotype than those from diagnosis or post-chemotherapy.
Conclusions: These findings highlight the importance of tracking CSC traits across disease stages and support the value of combining 2D live-cell imaging with 3D spheroid modeling to identify aggressive osteosarcoma subtypes and inform targeted therapies.

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